The effect of thermal stress on the physiology of two high-latitude corals from the environmentally variable intertidal and moderate subtidal habitats

by

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As the candidate's supervisor, I have approved this thesis/dissertation for submission.

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Preface

The research contained in this thesis was completed by the candidate while based in the Discipline of Biological Science, in the School of Life Sciences, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Westville, South Africa, from February 2020-July 2024 under the supervision of Dr David Glassom and co-supervision of Dr Dalene Vosloo. The research was financially supported by National Research Foundation (NRF) South Africa (grant number: MCR180627349036).

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate. Some of the methods used in this thesis are the same as that used by the candidate in her MSc and the paper that resulted from that, the re-use was inevitable.

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This thesis is presented in publication format. At the time of submission, Chapter 4 was published and presented at research symposia. Chapters 2, 3 and 5 are being prepared for submission. My role in each paper and presentation is indicated. The * indicates the corresponding author.

Chapter 2

*Boodraj, Prishani, David Glassom, and Dalene Vosloo. "Physiological responses of intertidal and subtidal corals *Anomastraea irregularis* and *Pocillopora verrucosa* exposed to experimental thermal stress" In preparation. Author contributions: Concept and design: PB and DG; Field and Laboratory work: PB; Analyses and writing of paper: PB; Editorial input: DG and DV.

Chapter 3

*Boodraj, Prishani, David Glassom, and Dalene Vosloo. "Antioxidant enzyme activity of intertidal and subtidal coral hosts *Anomastraea irregularis* and *Pocillopora verrucosa* exposed to experimental thermal stress" In preparation. Author contributions: Concept and design: PB, DG, DV; Laboratory work: PB; Analyses and writing of paper: PB; Editorial input: DV and DG.

Chapter 4

*Boodraj, Prishani and David Glassom. "The Effectiveness Of The 'Flexi-chamber' And 3D Photogrammetry To Measure Physiology Of Coral Fragments In The Field" Abstract ID: 12322. Presented to Twelfth Western Indian Ocean Marine Science Association (WIOMSA) Scientific Symposium, 10th to 15th October 2022, in Nelson Mandela Bay, South Africa. Presented by PB.

*Boodraj, Prishani and David Glassom. "The Effectiveness Of The 'Flexi-chamber' And 3D Photogrammetry To Measure Physiology Of Coral Fragments In The Field" Abstract ID: Presented to University of KwaZulu-Natal College of Agriculture, Engineering and Sciences Postgraduate Research Symposium (PRIS), 10th to 15th November 2022, in Durban, South Africa. Presented by PB.

*Boodraj, Prishani, David Glassom, and Dalene Vosloo. "Flexi-chambers and 3D photogrammetry effectively measure coral fragment physiology *in situ*." Journal of Experimental Marine Biology and Ecology 572 (2024): 151986 https://doi.org/10.1016/j.jembe.2024.151986. Author contributions: Concept and design: PB and DG; Field and Laboratory work: PB; Analyses and writing of paper: PB; Editorial input: DG and DV.

Chapter 5

*Boodraj, Prishani, David Glassom, and Dalene Vosloo. "The effect of reciprocal transplantation between an environmentally variable and moderate habitat on the physiology of corals *Anomastraea irregularis* and *Pocillopora verrucosa*" In preparation. Author contributions: Concept and design: PB and DG; Field and Laboratory work: PB; Analyses and writing of paper: PB; Editorial input: DG and DV.

Abstract

Corals are of great ecological and economic importance; however, they are increasingly threatened by mass bleaching events caused by ocean warming, which has become more frequent and is predicted to increase in frequency and severity in the coming years. The degree of coral bleaching, recoverability, and mortality is highly variable and considered to be affected by the coral's physiological and antioxidant capacity and the thermal regimes they experience. These mechanisms are still poorly understood and need to be investigated in different coral species from varying environmental regimes when exposed to prolonged thermal stress and during recovery to better inform conservation measures. Coral communities found on the subtropical East Coast of South Africa can be defined as 'extreme' due to the sub-optimum environmental conditions experienced especially in intertidal habitats and 'marginal' since, ecologically, they are not true accretive reefs. Climate change has not caused severe bleaching in corals of this region, but the physiological mechanisms that influence their thermal resilience/susceptibility are yet to be investigated. Therefore, this thesis examined the thermal resilience/susceptibility of *Anomastraea irregularis* (massive morphology) and *Pocillopora verrucosa* (branching morphology) from the intertidal and subtidal zones of the understudied rocky shores of Treasure Beach, east coast KwaZulu-Natal province, South Africa using both laboratory and field studies. The intertidal pools in this region are highly dynamic, with large summer daytime fluctuations of more than 10°C at spring tide while the subtidal zone is notably more environmentally stable.

These corals were maintained in closed recirculating aquaria and exposed to two constant thermal stress conditions (control: 26°C, thermal stress treatments: 28°C and 30°C) for three months and then were maintained at control conditions for two months to monitor recovery. Respiration, photosynthetic, and growth rates were measured monthly. The Symbiodiniaceae density, chlorophyll-a concentration, chlorophyll-a concentration per symbiont cell, lipid concentration, protein concentration, antioxidant enzyme activity (superoxide dismutase, catalase, glutathione peroxidase, glutathione s-transferase), and caspase 3 activity were analysed at the start and end of the thermal stress as well as at the end of the recovery period. The thermal stress treatments induced bleaching (significant decrease in Symbiodiniaceae density and chlorophyll-a concentrations) in both species from both habitats with associated significant decrease in photosynthetic and growth rates. Increased oxidative stress was also evident with the increased superoxide dismutase, catalase, glutathione peroxidase, and caspase 3 activity in

fragments in both thermal stress treatments at the start and end of thermal stress and at the end of recovery.

There were inherent physiological differences between the species and habitat that were maintained throughout the experiment, highlighting that variable thermal regimes and coral species can influence coral resilience to thermal stress. The intertidal corals were more resilient (less bleaching and fewer deaths) than their subtidal conspecifics, and *A. irregularis* appeared more resilient than *P. verrucosa* in both thermal stress treatments. The intertidal corals could lower their respiration rates to that of the control rates by the end of thermal stress and maintained higher Symbiodiniaceae densities, chlorophyll-a, and lipid concentrations than their subtidal conspecifics throughout the study. Resilience of *A. irregularis* may be a result of its thicker tissue, which allowed higher Symbiodiniaceae density and lipid concentrations and lower P:R ratios demonstrative of a more heterotrophic nature. Overall, higher protein concentrations and lower antioxidant enzyme activities (superoxide dismutase, catalase, and glutathione peroxidase) were evident in intertidal fragments than subtidal fragments and *A. irregularis* than *P. verrucosa* when thermally stressed. The higher protein concentrations may have facilitated the corals' physiological processes that made them more resilient to prolonged thermal stress. Therefore, these corals may have had lower antioxidant activities because of less oxidative stress. The results indicated much higher antioxidant activity in susceptible corals, suggesting that oxidative stress may be responsible for higher bleaching and mortalities. The photosynthetic and growth rates, Symbiodiniaceae density, chlorophyll-a concentration, and lipid concentration of both species from both habitats did not fully recover two months after thermal stress. Similarly, the antioxidant enzyme activities (superoxide dismutase, catalase, glutathione peroxidase), and caspase 3 activities of both species from both habitats did not decrease to control levels at the end of recovery, indicating that a longer period would be required for full recovery of the biochemical and physiological pathways of these corals. This has implications for coral reef recovery trajectories *in situ* since less time between mass bleaching events is predicted in the near future.

Field studies are important for validating the physiological responses found during laboratory studies since the static nature of laboratory experiments cannot account for the dynamic environmental conditions corals encounter *in situ*. A pilot study was conducted to determine if the 'flexi-chamber' and photogrammetry could be used to track respiration, photosynthesis, and growth of *A. irregularis* and *P. verrucosa* fragments (3 cm > 4 cm) in the intertidal and subtidal habitats in Park Rynie, east coast KwaZulu-Natal province, South Africa. This study found that

the two methods could be optimised to effectively measure the physiological processes of coral fragments in both habitats at relatively low cost and low complexity.

To understand the physiological responses of these species when exposed to thermal stress in their natural habitats an *in-situ* experiment at Treasure Beach was conducted during the warmer austral spring and summer months where fragments of both species were reciprocally transplanted between the two habitats. Controls were established by placing fragments in their original habitats. Respiration, photosynthesis, growth rates, and coral health scores were measured monthly for six months. The physiological rates were measured using the optimised methods that were obtained during the pilot study. The Symbiodiniaceae density, chlorophyll-a concentration, chlorophyll-a concentration per symbiont cell, and lipid concentration were also analysed at the start and end of the study. Similar to the laboratory results, differential physiological responses between species and habitat were evident in response to the reciprocal transplantation. Intertidal *A. irregularis* and *P. verrucosa* transplanted into the subtidal habitat showed potential acclimation to the subtidal habitat. These fragments were able to adjust their P:R ratios and maintain higher Symbiodiniaceae cell density, Symbiodiniaceae chlorophyll-a concentration, and Symbiodiniaceae chlorophyll-a concentration per cell, and lipid content thereby experiencing less bleaching and mortalities. *Anomastraea irregularis* appeared more physiologically plastic (altering respiration rates and maintaining higher Symbiodiniaceae cell density and lipid concentration) and therefore more tolerant (less bleaching and mortalities) than *P. verrucosa* to the changes in environmental conditions.

The laboratory and field results add to the limited knowledge of how high-latitude corals of different species and from habitats of differing environmental regimes react physiologically to long-term thermal stress. The results are promising since these resilient corals may be used in future conservation initiatives. The results of this thesis show the ability of some coral species to acclimatise and/or adapt to different environmental conditions, however, the potential for these corals to acclimatise/adapt to global climate change related stressors still warrants further investigation especially since several other stressors are also impacting reef systems.

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The onset of the Covid-19 pandemic in March 2020, just a month after I enrolled, presented significant challenges. With people working from home, obtaining necessary equipment and permits was greatly delayed. I also endured the Durban riots, where I resided opposite a mall that was constantly set ablaze, then there was the flooding which I had to brave to switch on the electricity to get power to my tanks. Tragically, I also lost a few family members during this period. These events took a major mental toll on me, and I lost sight of the importance of a Ph.D. in biological science in the grand scheme of things. So, I acknowledge the NRF bursary for motivating me to complete this PhD, as the alternative of owing the state money I cannot repay was unacceptable.

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Chapter 1: Introduction

1.1 Background

Extant scleractinian reef-building corals developed over the last 24 million years ago (Bellwood et al. 2017) and are referred to as ecosystem engineers as they are responsible for promoting and maintaining the biological complexity and diversity of the coral reef ecosystems by depositing their calcium carbonate ($CaCO_3$) structural framework (Hoegh-Guldberg 1999; Williams and Graham 2019). Coral reefs are mainly found between 30°N and 30°S in the tropical and subtropical waters around the equatorial belt at depths from 0-30 m (Allemand and Osborn 2019). Coral reefs are immensely valuable ecosystems biologically, ecologically, and socio-economically at a global scale (Spurgeon 1992; Spalding et al. 2001; Woodhead et al. 2019). Coral reefs only cover approximately 0.2% of the seafloor but house approximately 830 000 species (Fisher et al. 2015) and support half a billion people globally (Bellantuono et al. 2012a). They have been estimated to provide ecosystem services worth $375 billion per year, especially from tourism and fisheries (Bellantuono et al. 2012a).

The coral animal, a variety of microscopic symbionts, including viruses, algae, and bacteria, are collectively called the coral holobiont (Stat et al. 2012). The symbiotic relationship between the coral hosts and unicellular photosynthetic dinoflagellates belonging to the family Symbiodiniaceae (formerly known as zooxanthellae) in particular (LaJeunesse et al. 2018), enables corals to thrive in nutrient-poor or oligotrophic waters (Muscatine & Porter 1977, Muscatine 1990, Lesser 2004, Muller-Parker et al. 2015). This symbiosis is thus the basis for the high productivity found in coral reef ecosystems (Brown 1997; Stat et al. 2012). Therefore, the coral host-algal endosymbiont relationship is vital to the functioning of coral reef ecosystems and the ecological and socio-economic services they provide (Allemand and Osborn 2019).

Symbiodiniaceae are housed in the endodermal tissue of the coral host within intracellular vacuoles called symbiosomes, believed to come from the plasma membrane of the host cells during the initial acquisition of the algae (Shinzato et al. 2014). The coral hosts provide shelter from herbivorous animals and inorganic nutrients (the waste products of coral metabolism) to the Symbiodiniaceae. In return, the Symbiodiniaceae provide the corals with carbohydrates that aid in the high rate of $CaCO_3$ deposition for the coral skeletons and oxygen for coral respiration (Muscatine and Porter 1977; Dubinsky and Jokiel 1994; Lough 2008; Osinga et al. 2012). Symbiodiniaceae can also provide the corals several other biomolecules such as

glycerol, organic acids, amino acids, lipids, and polyunsaturated fatty acids (Yellowlees et al. 2008; Stambler 2011). The Symbiodiniaceae can provide up to 95% of the coral's daily energy requirements; however, the corals are also edacious heterotrophs (Houlbrèque and Ferrier-Pagès 2009). The dependence of heterotrophy to fulfil a coral's daily energy requirement does vary between coral species and the environmental conditions they experience (Anthony and Fabricius 2000; Houlbrèque and Ferrier-Pagès 2009). There is no evidence that a coral can survive on either heterotrophic or photosynthetic carbon alone, because both processes are important for the assimilation of energy for daily metabolic requirements (Grottoli et al. 2006; Palardy et al. 2008; Houlbrèque and Ferrier-Pagès 2009). Although the coral-algal symbiosis is crucial to the success of scleractinian reef-building corals, it also makes them vulnerable to changes in environmental conditions such as changes in turbidity, temperature, pH and salinity (Baker et al. 2008; Wooldridge 2010).

1.2 Threats to corals and coral bleaching

Declines in scleractinian reef-building coral cover have been linked to various local anthropogenic activities such as destructive fishing practices, coastal development, sedimentation, intense tourism and increasing pollution (Carpenter et al. 2008; Alvarez-Filip et al. 2011; Hoegh-Guldberg et al. 2018; Lamb et al. 2018; Wolff et al. 2018; Andrello et al. 2021; Nama et al. 2023). However, global-scale ocean warming and acidification due to anthropogenically induced climate change have been identified as the main existential threats to corals around the world (Hughes et al. 2003; Pandolfi et al. 2011; Anthony 2016; Bruno and Valdivia 2016; Hughes et al. 2017b; Kamenos and Hennige 2024). Corals live close to their upper thermal limits, which means that an increase in sea surface temperature (SST) of even 1-2°C above the mean summer maximum for an extended period (≥ four weeks) may be very detrimental to the coral-algal symbiosis and may likely result in a significant bleaching event (Lesser 1997; Eakin et al. 2009; Richards et al. 2015).

Reef-building corals respond to the existential threat of ocean warming and other stressors, such as prolonged exposure to solar radiation, bacterial infection, salinity stress, sedimentation, and cold shock by bleaching (Brown 1997; Lesser 1997; Fujise et al. 2014). Coral bleaching occurs when corals expel their endosymbiotic dinoflagellate algae from their tissue or there is a loss in the photosynthetic pigments of the algae resulting in the corals ultimately becoming white (Richmond 1993; Brown 1997; Hoegh-Guldberg 1999; Fujise et al. 2014; Ainsworth et al. 2016). Coral mortality usually follows bleaching because of the reduced energy received from the algal symbionts, which further leads to reduced growth, defence, and healing ability

(Baker et al. 2008; Anthony et al. 2009). However, if suitable environmental conditions resume, corals can recover from bleaching. Symbiont communities will slowly repopulate the host through cell division *in hospite* and/or through uptake from the environment (Toller et al. 2001).

Coral bleaching was not widely recognised until the early 1980s, but since then the phenomenon has occurred more frequently and has resulted in declines of approximately 30-50% of coral cover worldwide (Brown 1997; Baker et al. 2008; Silverstein et al. 2015; Ainsworth et al. 2016; Tebbett et al. 2023). Global coral bleaching events of 1998, 2010, 2014-2017, and 2023-2024 were linked to rising SST primarily caused by human-induced climate change (Hughes et al. 2017b; Cziesielski et al. 2019; Eakin et al. 2019; Reimer et al. 2024). In addition to climate change, climatic cycles such as the El Niño-Southern Oscillation (ENSO) events cause marine heatwaves and can also exacerbate bleaching events by causing reduced cloud cover, higher temperatures and atmospheric pressures leading to greater thermal stress on corals (McGowan and Theobald 2017; Claar et al. 2018; Vargas-Ángel et al. 2019; Zhang et al. 2023). The extensive loss of functioning coral reefs that follow mass bleaching events attests to the severe consequences that result from the breakdown of the coral-algal symbiosis (Hughes et al. 2017a). These global coral bleaching events have raised the question of whether functioning reef ecosystems can still exist by the end of this century (van Oppen et al. 2015; Hoegh-Guldberg et al. 2018), especially since the frequency and severity of these events are expected to increase with the predicted rise in SST and ENSO events over the coming century (Frieler et al. 2013; van Oppen et al. 2015; Osborne et al. 2017; Camp et al. 2018a; Feng et al. 2023; IPCC 2023).

Coral bleaching from heat stress is widely thought to result from initial damage to Symbiodiniaceae cells rather than coral tissue (Lesser 1997; Berkelmans and van Oppen 2006; Strychar and Sammarco 2009; Cunning and Baker 2012; Fujise et al. 2014; Safuan et al. 2021; Doering et al. 2023). Heat-induced bleaching causes damage to the D1 reaction centre protein of PSII (Warner et al. 1999). Excessive heat also causes damage to the lipid structures in the thylakoid membrane of the chloroplasts in the Symbiodiniaceae cells which leads to heat-dependent photoinhibition (Lesser 1997; Takahashi et al. 2004; Tchernov et al. 2004). This causes excessive production of reactive oxygen species (ROS), which further damage Symbiodiniaceae lipid structures producing more ROS in a positive feedback loop (Weis 2008). The ROS also damages deoxyribose nucleic acid (DNA), protein and lipid structures of host coral cells (Lesser 1997; Weis 2008). Thus, corals expel the Symbiodiniaceae to avoid

mortal damage (Lesser 1997; Weis 2008). This eventually leads to mortality due to the energy deficit in the bleached corals, as less energy is being transferred by the endosymbiont algae and an increase in energetic costs due to stress responses (Brown 1997; Grottoli et al. 2014).

However, literature has shown that coral responses to thermal stress have been highly variable both taxonomically and geographically (Hughes et al. 2003; Dove et al. 2006; Baker et al. 2008; Guest et al. 2012; Sweet and Brown 2016; Sully et al. 2019; Kuo et al. 2023). There have been several accounts of bleached and healthy corals being near each other (Berkelmans and Willis 1999; Hughes et al. 2003; Humanes et al. 2022). Several studies show that the morphology of the corals may affect their resilience/susceptibility to bleaching when exposed to thermal stress (Marshall and Baird 2000; Loya et al. 2001; Zawada et al. 2019b). Branching genera such as *Acropora* and *Pocillopora* which have higher algal cell densities and are more dependent on receiving energy from their endosymbiont algae, are considered to be more susceptible to bleaching (Coles and Jokiel 1977; Loya et al. 2001; Guest et al. 2012; Levas et al. 2013), while massive genera such as *Favia* and *Favites* house lower densities of algae and may receive more of their energy through heterotrophy, therefore being less prone to bleaching (Coles and Jokiel 1977; Loya et al. 2001; Guest et al. 2012; Levas et al. 2013). However, this relationship is more complex than that, and factors such as the tissue thickness and metabolism due to the morphology also influence the corals' resilience/susceptibility to bleaching (Gates and Edmunds 1999; Grottoli et al. 2017; Carballo-Bolaños et al. 2019).

Resilience takes into account both resistance to and recovery from a stressor, and therefore it is important to also investigate the recovery of bleached corals when suitable conditions return (Visram and Douglas 2007; McLachlan et al. 2020). A lot of research mainly focuses on resistance to bleaching and rarely looks at the recovery trend, which is an active evolutionary adaptation (McLachlan et al. 2020). For example, repair mechanisms to the photosystem (PS) II proteins of the Symbiodiniaceae can counteract heat stress damage to the algal cells (Takahashi et al. 2004). Therefore, a measure of bleaching tolerance can be attained by comparing the rate of PSII repair to the rate of damage (Takahashi et al. 2004). Corals have numerous antioxidant enzymes and proteins that work to reduce/repair ROS cellular damage, thereby limiting bleaching (Downs et al. 2002; Yakovleva et al. 2004). Therefore, the differential antioxidant potential of corals may also be responsible for the observed variable bleaching responses. Symbiodiniaceae genotypes have varying heat tolerances (Baker 2003; LaJeunesse et al. 2018), and Symbiodiniaceae of the same genotype from different latitudes or habitats may also have varying tolerances and photochemical performance (Howells et al.

2012). This can contribute to the observed variability in coral bleaching responses (Howells et al. 2012). Corals that host thermally tolerant Symbiodiniaceae genotypes may be better equipped to endure elevated SST (Császár et al. 2010; Baker et al. 2015; Howells et al. 2020; Hume et al. 2020; Turnham et al. 2023; Alessi et al. 2024). Bleached corals may be recolonised by more thermally tolerant Symbiodiniaceae genotypes, but, the adaptive value of this is debated (Hoegh-Guldberg et al. 2002) and it does not occur in all cases (Visram and Douglas 2007). The microbial community of corals can also rapidly shift in response to thermal stress thereby enhancing thermal tolerance of the corals (Ziegler et al. 2017; Epstein et al. 2019). Some bleached coral hosts may catabolise lipid reserves, carbohydrates, and proteins, and increase their heterotrophy to survive and recover from the heat stress (Connolly et al. 2012; Levas et al. 2013; Bauman et al. 2014; Levas et al. 2016). The coral holobiont resilience/susceptibility is determined by all partners of the coral holobiont, and the complexity of the symbiotic relationships makes it difficult to predict future responses to climate change without rigorous research involving all aspects of the coral holobiont (Bellantuono et al. 2012b; Stat et al. 2012; Cziesielski et al. 2019).

Thermal regimes have been found to also influence coral resilience/susceptibility to thermal stress. Extreme habitats are characterised as having sub-optimal, highly fluctuating environmental conditions (e.g. temperature, pH, aragonite/calcite saturation state, light, oxygen, turbidity, nutrients, salinity) over daily/tidal/weekly/seasonal time scales (Schoepf et al. 2023). Corals that exist in extreme habitats like intertidal rock pools, back-reef pools, mangrove lagoons, and intertidal reefs may be better acclimatised or adapted to thermal stress and are therefore more resilient to anomalous temperatures than counterparts that exist in environmentally moderate habitats (Oliver and Palumbi 2011; Sawall et al. 2015; Morgan et al. 2017; Safaie et al. 2018; Jung et al. 2021; Keshavmurthy et al. 2021; Marhoefer et al. 2021; McLachlan et al. 2021; Scucchia et al. 2023; Speelman et al. 2023; Brown et al. 2024; Huang et al. 2024). Corals from extreme habitats may be at the edge of their environmental limits and therefore experience selective pressures that result in unique genetic and physiological adaptations and sometimes association with heat-tolerant Symbiodiniaceae to allow them to persist in sub-optimal conditions (Oliver and Palumbi 2011; Camp et al. 2019; Jung et al. 2021; Marhoefer et al. 2021; Haydon et al. 2023; Scucchia et al. 2023; Huang et al. 2024). However, the resilience to thermal stress in corals from extreme habitats may be species-specific (Camp et al. 2019; Klepac and Barshis 2020; Klepac and Barshis 2022). Furthermore, there are cases where temperature variability had no or negative effects on coral thermal resilience (Schoepf

et al. 2019; Klepac and Barshis 2020; Schoepf et al. 2021; Klepac and Barshis 2022). The physiological mechanisms behind the disparity in resilience/susceptibility thermal tolerance of corals from extreme habitats are still unclear and warrants further investigation. Marginal coral communities such as those found at high latitudes are also worth investigating as they may possess resilient traits since they have different ecological functions than typical tropical reefs (Keshavmurthy et al. 2021; Schoepf et al. 2023). Furthermore, conditions in extreme and marginal habitats may match those expected under climate change so they are of particular importance because they can provide insight into how corals from tropical reefs may respond to future climate scenarios (Schoepf et al. 2023). However, corals adapted to extreme and marginal habitats may also be vulnerable to predicted climate change since the already sub-optimal conditions would be intensified (Scucchia et al. 2023).

The high variability in corals' resilience/susceptibility to thermal stress highlights the need to investigate the specific physiological, cellular, and molecular mechanisms that drive these response (Fox et al. 2019; Rinkevich 2019). Determining the factors that drive the resilience/susceptibility of different coral species from variable environmental conditions and different latitudes can aid in predicting corals' adaptability to climate change and conservation initiatives (Camp et al. 2019; Schoepf et al. 2023). Performing reciprocal coral transplant experiments between extreme and moderate habitats may provide an additional way to understand the complex responses of corals since the environmental variables are not static and naturally fluctuate as opposed to conventional laboratory studies (Oliver and Palumbi 2011; Mayfield et al. 2012; Safaie et al. 2018). Some scientists also believe that climate change is outpacing the natural adaptive capacity of corals and that, in addition to conventional restoration techniques, manipulative interventions aimed at either the coral host or its symbionts are required (van Oppen et al. 2017; Howells et al. 2021; Quigley 2024). Understanding the physiological mechanisms that drive resilience/susceptibility to heat stress in understudied high-latitude natural stock corals of different species of differing morphology from environmentally variable and moderate habitats would provide baseline information for conventional conservation and assisted evolution initiatives.

1.3 Physiological and biochemical responses to thermal stress

Corals are sedentary animals, hence, they cannot simply migrate to new environmental optima, and therefore, understanding their stress responses is vital to their conservation (Palumbi et al. 2014). Despite basic knowledge of the mechanism of bleaching, many questions remain about the physiological, cellular and molecular drivers of bleaching and the genetics that underlie

these drivers due to the complexity of the coral holobiont and variability in its response to a given stressor (Sweet and Brown 2016). Measuring the effects of heat stress on the physiology of corals is still important to quantify the link between changes in gene expression and changes in function (Cziesielski et al. 2018; Cziesielski et al. 2019). Understanding physiological responses is also important for validating proteomic and metabolomic level observations (Sogin et al. 2016; Cziesielski et al. 2018). Depending on inherent traits of coral physiology like metabolic rates, energy reserves, antioxidant potential, and Symbiodiniaceae phenotypic plasticity, pre-peak temperatures may either enhance resilience at peak temperatures or cause susceptibility (Downs et al. 2000; Grottoli et al. 2014; Safaie et al. 2018). These quantifiable responses are, therefore, insightful biomarkers in coral thermal stress studies.

Coral physiological research frequently examines respiration and photosynthetic rates, as these fundamental metabolic processes of the coral holobiont are key drivers of the coral host's skeletal growth (e.g. Al-Horani et al. 2003; Kenneth and Erez 2006; Anthony et al. 2008) and can rapidly indicate sub-lethal stress responses (Lesser 2013). For example, when *Acropora millepora* were exposed to low pH, changes in respiration and photosynthetic rates were observed before any detectable changes in calcification were evident (Kaniewska et al. 2012). An increase in the respiration rate of an organism can indicate acute stress, conversely, a decrease in respiration rate can be indicative of acclimation or metabolic depression as a result of a stressor (Guppy and Withers 1999). Bleaching due to increased SST has been found to cause increased respiration rates in some corals (e.g. Coles and Jokiel 1977; Borell et al. 2008; Levas et al. 2013) and reduced respiration rates in others (e.g. Rodrigues and Grottoli 2007). After a month of recovery from increased SST, some bleached corals were found to have reduced photosynthetic rates compared to pre-bleaching rates due to either decreased chlorophyll-a content or algal densities or a combination of the two (e.g. Rodrigues and Grottoli 2007; Borell et al. 2008; Levas et al. 2013). Borell et al. (2008), showed the importance of heterotrophy to the maintenance of gross photosynthesis in *Stylophora pistillata* colonies exposed to heat stress, which also highlights the importance of providing corals with a food source when researching the bleaching responses of corals when exposed to thermal stress.

The P:R (gross photosynthetic rate: respiration rate) ratio has been used to determine if a coral can survive on the energy obtained from its endosymbiotic algae (Coles and Jokiel 1977; Lesser 2013; Muller-Parker et al. 2015). A ratio less than 1 indicates that the coral received insufficient energy from the Symbiodiniaceae whilst a ratio greater than 1.5 indicates that the coral received abundant energy from the Symbiodiniaceae (Baker et al. 2015). The significant reduction in

Symbiodiniaceae chlorophyll-a concentration or Symbiodiniaceae cells associated with bleaching usually results in reduced photosynthesis, which in turn leads to a P:R ratio of less than 1 in most bleached corals (e.g. Coles and Jokiel 1977; Rodrigues and Grottoli 2007). Reduced photosynthesis and enhanced respiration indicative of increased heterotrophy have been seen as a mechanism to survive sub-optimal variable environmental conditions in mangrove habitats (e.g. Camp et al. 2017; Camp et al. 2019). The P:R ratio of some reef flat corals were found to be maintained even under extreme physiochemical conditions (Gruber et al. 2017). Corals from extreme habitats that can adjust their metabolic rates were seen to be more resilient to thermal stress (Camp et al. 2019). Some intertidal corals were more tolerant to thermal stress by maintaining lower metabolism than subtidal corals, thereby reallocating energy for non-essential activities towards immunity and cellular repair activities (Huang et al. 2024).

The Symbiodiniaceae cell density and chlorophyll-a concentration should also be quantified as it can be used to infer responses to stressors (Muscatine 1990; Nystrom et al. 2001). For example, a reduction in Symbiodiniaceae density is associated with thermal stress, whilst a reduction in chlorophyll-a concentration is associated with environmental stressors such as turbidity, sedimentation and increased ultra-violet radiation (Jones 1997; Hoegh-Guldberg et al. 2007). It is also important to identify the specific Symbiodiniaceae genotypes present in the corals being investigated, as there is evidence that genotypes respond differently to thermal stress and can influence the metabolic processes in the coral host (Cunning et al. 2015; Drury et al. 2017; Camp et al. 2019; McLachlan et al. 2020). Enhanced thermal tolerance in some coral species was attained by acquiring stress-resistant Symbiodiniaceae species *Durusdinium* spp. and *Cladocopium* spp. (e.g. Berkelmans and van Oppen 2006; Stat and Gates 2011; Levas et al. 2013; Hume et al. 2020). Thermally variable habitats can also enhance thermal resistance in corals hosting the same symbiont type (e.g. Schoepf et al. 2015b; Jung et al. 2021).

The growth of the coral host skeleton is an important parameter due to its implication for coral reef ecosystem functioning (Harborne et al. 2017). It can also give a measure of sub-lethal response to environmental stressors. Some coral species decrease their growth during bleaching due to the energy deficit experienced (e.g. Suzuki et al. 2003; Carilli et al. 2009; Colombo-Palotta et al. 2010; Levas et al. 2013; Foster et al. 2014; Razak et al. 2020; Kramer et al. 2022), some may halt growth completely (e.g. Goreau and Macfarlane 1990) and others can resume their pre-bleaching growth rates as soon as chlorophyll-a concentrations and energy reserves

are replenished (e.g. Mendes and Woodley 2002). The growth rates of corals from extreme habitats are usually reduced as a trade-off for surviving sub-optimal environmental conditions (Camp et al. 2019). However, no change in the growth rates was observed in some reef flat corals exposed to elevated temperatures (Dandan et al. 2015). Quantifying coral growth has proven challenging owing to the morphological complexity of coral colonies and the variable growth patterns of individual species (Buddemeier and Kinzie III 1976; Veal et al. 2010; Herler and Dirnwöber 2011). However, with technological advances, three-dimensional (3D) photogrammetry appears to be a low-cost, user-friendly and efficient method that can capture the structural complexities of diverse coral morphologies over a period of time (Figueira et al. 2015; Lavy et al. 2015; Ferrari et al. 2016; Guo et al. 2016; Ferrari et al. 2017; Lange and Perry 2020).

The health status of corals can be determined by analysing their energy reserves which consists of lipids, carbohydrates, and proteins (Rodrigues and Grottoli 2007). The concentrations of these macromolecules are highly specific to species, geography, and environmental regimes (Keister et al. 2023). Lipids and proteins are important energy reserves that are utilised by some coral species when they experience energy deficits during stressful periods and can be vital in the resilience of corals when exposed to heat stress (Grottoli et al. 2006; Rodrigues and Grottoli 2007; Imbs and Yakovleva 2012; Levas et al. 2013; Schoepf et al. 2015b; Zhang et al. 2024). Monitoring the changes in the energy reserves and tissue biomass of corals exposed to heat stress can thus allow us to gain insight into the corals' sub-lethal responses and how the responses contribute to the corals' resilience/susceptibility to the stress (Baird and Marshall 2002; Grottoli et al. 2006; Rodrigues and Grottoli 2007; Borell et al. 2008; Anthony 2016; McLachlan et al. 2020).

The efficiency to synthesise and utilise energy reserves as an alternative energy source during thermal stress may also be species-specific and may depend on environmental regimes (Rodrigues and Grottoli 2007; Levas et al. 2013; Wall et al. 2019; Jung et al. 2021). For instance, species like *Montipora capitata* that increase their feeding during bleaching and recovery from thermal stress may be more adept at maintaining and restoring energy reserves (Grottoli et al. 2006; Rodrigues and Grottoli 2007) than species like *Porites compressa* that are dependent on photosynthetically derived energy alone (Rodrigues and Grottoli 2007). Thermally susceptible subtidal corals were observed to mainly utilise energy-poor carbohydrate stores during peak thermal stress, while more resilient intertidal corals could maintain energy reserves (Schoepf et al. 2015a; Jung et al. 2021). Proteins also play an integral

role in various biological functions, and there are several specialised proteins in corals that can contribute to adaptation to environmental stressors, colour expression, and skeleton formation (Downs et al. 2000; Voolstra et al. 2011; Seveso et al. 2019; Mummadisetti et al. 2021; Satoh et al. 2021; Tisthammer et al. 2021). Therefore, coral species with higher protein concentrations are seen to be more resilient to thermal stress as they are better equipped to mount biochemical defences, which helps them resist/repair cellular damage and maintain homeostasis (e.g. Barshis et al. 2010; Carballo-Bolaños et al. 2019).

Biochemical defences in response to thermal stress can include elevated activity of antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase to neutralise ROS to reduce cellular damage (e.g. Downs et al. 2002; Yakovleva et al. 2004; Baird et al. 2009; Hawkins et al. 2015). These enzymes are usually a short-term defence mechanism, and beyond a certain ROS concentration threshold, the antioxidant enzyme production systems can be overwhelmed, and significant oxidative damage occurs to essential cellular structures (e.g. Downs et al. 2000; Yakovleva et al. 2004; Baird et al. 2009; Fitt et al. 2009; Wall et al. 2018; Dias et al. 2019b; Tisthammer et al. 2021; Doering et al. 2023). This leads to increased caspase 3 activity which signals programmed cell death/apoptosis (e.g. Ainsworth et al. 2008; Kvitt et al. 2016; Yu et al. 2017; Thummasan et al. 2021) and increased lipid peroxidation (e.g. Lesser 1997; Downs et al. 2002; Rodrigues and Grottoli 2007). Coral species have been found to have differential antioxidant activities in response to thermal stress, and environmental regimes also seem to influence the corals' antioxidant potential. Corals that are susceptible to thermal stress are considered to have lower ROS thresholds and have been observed to promptly elevate antioxidant enzyme activities to mitigate ROS (e.g. Downs et al. 2002; Griffin et al. 2006; Krueger et al. 2015; Dias et al. 2019a; Huang et al. 2024). However, antioxidant enzyme activities were also observed to rapidly increase in resilient corals from extreme habitats (e.g. Flores-Ramirez and Linan-Cabello 2007; Fitt et al. 2009; Tisthammer et al. 2021; Wang et al. 2022; Zhai et al. 2024). Therefore, it is essential to investigate the antioxidant potential of corals in conjunction with physiological parameters to fully understand the corals' bleaching trajectories (Dias et al. 2020).

1.4 Justification

Scleractinian reef-building corals now face several local and global stressors at once (Franca et al. 2020). More frequent and severe coral bleaching and related mortalities are predicted in the near future, with chronic ocean warming and associated increases in the frequency and intensity of marine heat waves (Hoegh-Guldberg et al. 2023). The loss of corals will cause a

considerable loss of marine biodiversity and biomass, truncated food webs and deteriorating water quality (Hoegh-Guldberg et al. 2018; Smale et al. 2019). However, the highly dynamic responses of corals to thermal stress across species, environmental regimes, and latitudes are integral to the persistence of reefs and leave room for further investigation as they provide avenues to conserve coral reefs (van Oppen et al. 2015; Rinkevich 2019; McClanahan et al. 2020a; McClanahan et al. 2020b; Schoepf et al. 2023). Identifying resilient corals and the mechanisms that make them resilient from habitats that potentially harbour stress-tolerant alleles is primarily necessary for reef conservation and restoration efforts, given the current rates of ocean warming (van Oppen et al. 2015; Camp et al. 2019; Thomas et al. 2022).

According to the Schoepf et al. (2023) conceptual framework, high-latitude coral communities on the subtropical East Coast of South Africa can be categorised as 'extreme' since they persist in sub-optimum environmental conditions and 'marginal' because ecologically, they do not form true accretive reefs (Schleyer et al. 2018). South African corals are close to the southern limits of coral distribution and corals in the iSimangaliso Wetland Park in the northern limits of the province of KwaZulu-Natal exhibited minimal bleaching from climate change (Schleyer et al. 2018; Porter et al. 2021). However, the underlying physiological mechanisms that drive this bleaching trend have yet to be thoroughly investigated in these corals or corals in non-reef habitats found further south of the province of KwaZulu-Natal. Several coral species of varying growth forms inhabit the environmentally variable intertidal and moderate subtidal zones of the rocky shores of Treasure Beach, east coast KwaZulu-Natal province, South Africa (Smit 2014). The intertidal pools are characterised as being highly dynamic, with large summer daytime fluctuations of more than 10°C at spring tide, while the subtidal zone is more environmentally stable (Smit and Glassom 2017). *Anomastraea irregularis* (massive morphology) and *Pocillopora verrucosa* (branching morphology) are common species found thriving in both zones (Smit 2014). Smit (2014) found differential physiological responses in zooxanthellae density, chlorophyll-a and lipid content between the species in response to seasonal change in the intertidal pools. *Pocillopora verrucosa* exhibited more changes in zooxanthellae density, chlorophyll-a and lipid content throughout the year than *A. irregularis* (Smit 2014). *Anomastraea irregularis* from both habitats were found to be more thermotolerant, exhibiting less genetic change than *P. verrucosa* from both habitats when exposed to short-term experimental thermal stress (Onyango 2020). Furthermore, upregulation in genes associated with protein synthesis in both species from the intertidal habitat were found to confer higher thermotolerance than the subtidal conspecifics (Onyango 2020). These

findings highlighted the possibility of species and habitat-specific physiological acclimatisation and/or adaptations in these corals to mediate thermal stress. Therefore, this thesis attempted to gain a deeper understanding of the physiological responses that influence the resilience/susceptibility of the unique corals found in non-reef habitats in the KwaZulu-Natal province. Measuring the physiological responses of these corals in response to thermal stress will aid in quantifying the link between change in gene expression and change in function (Cziesielski et al. 2018; Cziesielski et al. 2019). Long-term thermal stress studies incorporating understudied high-latitude South African corals, especially in extreme intertidal habitats, contributes valuable information to build a more comprehensive catalogue of coral responses to thermal stress (McLachlan et al. 2020).

1.5 Aim/Objectives

Therefore, the aim of this thesis was to investigate the physiological mechanisms that influence the thermal resilience/susceptibility of *Anomastraea irregularis* (massive morphology) and *Pocillopora verrucosa* (branching morphology) from the environmentally variable intertidal pools and moderate subtidal habitat of Treasure Beach, South Africa.

This was achieved by conducting a long-term laboratory experiment exposing the two species from the two habitats to three months of thermal stress and then two months of recovery and measuring their physiological responses i.e., respiration, photosynthetic, and growth rates as well as change in tissue composition (Symbiodiniaceae density, Symbiodiniaceae chlorophyll-a, and lipid concentrations), antioxidant activity (superoxide dismutase, catalase, and glutathione peroxidase) and caspase 3 activity. A field study was also conducted during six months of summer, where fragments of both species were reciprocally transplanted between habitats and controls were established by transplanting fragments in their original habitats reciprocally, with selected physiological rates measured monthly.

Objectives:

1) To measure specific physiological and biochemical responses and compared them between control corals and those exposed to varying degrees of environmentally relevant thermal stress.

2) To evaluate the potential of the ‘Flexi-chamber’ and photogrammetry to track the physiology of coral fragments in the field.

3) To reciprocally transplant *Anomastraea irregularis* and *Pocillopora verrucosa* fragments between intertidal and subtidal habitats during warmer months and measure specific physiological responses of the corals and compare them between the control and transplants.

1.6 Outline of the thesis structure

This thesis is written in the format of a series of papers. As such, related chapters may have some sections of their methods and materials being similar, but all references were provided at the end of the thesis.

Chapter 1: Introduction of the research topic and a literature review summary.

Chapter 2: A laboratory experiment to determine the physiological responses that influence the resilience/susceptibility of *Anomastraea irregularis* and *Pocillopora verrucosa* fragments from intertidal and subtidal habitats when exposed to varying levels of increased temperatures.

Chapter 3: An outline of the biochemical responses that influence the resilience/susceptibility of *Anomastraea irregularis* and *Pocillopora verrucosa* fragments from intertidal and subtidal habitats when exposed to varying levels of increased temperatures.

Chapter 4: A pilot study outlining the effectiveness of the 'flexi-chamber' and 3D photogrammetry methods to track the physiology of coral fragments in the field. The effectiveness of the flexi-chamber to track photosynthesis and respiration and the 3D photogrammetry method to measure the growth of small individual coral fragments was assessed.

Chapter 5: An outline of the reciprocal transplantation field experiment to understand the physiological responses that influence the resilience/susceptibility of *Anomastraea irregularis* and *Pocillopora verrucosa* fragments *in situ*.

Chapter 6: Overall discussion/recommendations and conclusion of the thesis.

Chapter 2: Physiological responses of intertidal and subtidal corals *Anomastraea irregularis* and *Pocillopora verrucosa* exposed to experimental thermal stress

2.1 Abstract

Understanding the effects of thermal stress on the physiology of corals is vital for their conservation during the Anthropocene. This study examined the thermal resilience/susceptibility of *Anomastraea irregularis* (massive morphology) and *Pocillopora verrucosa* (branching morphology) collected from environmentally variable intertidal and more stable subtidal habitats from the east coast of South Africa (29°57'32.8"S 30°59'19.0"E). These corals were maintained in closed recirculating aquaria and exposed to two thermal stress conditions (control: 26°C, thermal stress treatments: 28°C and 30°C) for three months and then were maintained at control conditions for two months to monitor recovery. Rates of respiration, photosynthetic, and growth were measured monthly. The Symbiodiniaceae density, chlorophyll-a concentration, chlorophyll-a concentration per symbiont cell, and lipid concentration were analysed at the start and end of the thermal stress and at the end of the recovery period. The thermal stress treatments induced bleaching (significant decrease in Symbiodiniaceae density and chlorophyll-a concentrations) in both species from both habitats with an associated significant decrease in photosynthetic and growth rates. There were inherent physiological differences between the species and habitat that were maintained throughout the experiment, highlighting that extreme habitats and coral species can influence coral resilience to thermal stress. The intertidal corals were more resilient (less bleaching and death) than their subtidal conspecifics, and *A. irregularis* appeared more resilient than *P. verrucosa* in both thermal stress treatments. The intertidal corals were able to lower their respiration rates to that of the control rates by the end of thermal stress and maintained higher Symbiodiniaceae densities, chlorophyll-a, and lipid concentrations than their subtidal conspecifics throughout the study. The resilience of *A. irregularis* may be a result of its thicker tissue which allowed higher Symbiodiniaceae density and lipid concentrations and lower P:R ratios demonstrative of a more heterotrophic nature. While there was some recovery, the photosynthetic and growth rates, Symbiodiniaceae density, chlorophyll-a concentration, and lipid concentration of both species from both habitats did not fully recover two months after thermal stress, indicating that a longer period would be required for full recovery of these corals. This study adds to the limited knowledge of how high-latitude corals of different morphology and from habitats of differing environmental regimes react and recover physiologically to long-term experimental thermal stress.

Keywords: respiration, photosynthesis, bleaching, lipid, Symbiodiniaceae

2.2 Introduction

Coral symbiosis with photosynthetic algae, Symbiodiniaceae, is vital to the productivity of the corals and, by extension, the reef ecosystems (Brown 1997; Stat et al. 2012; Muller-Parker et al. 2015). However, this symbiosis can disintegrate when the corals encounter biological or environmental stressors (Baker et al. 2008; Wooldridge 2017). Coral bleaching is the resulting phenomenon when the corals eject the Symbiodiniaceae from their tissue, or there is a loss in the photosynthetic pigments of the Symbiodiniaceae in response to stress (Baird et al. 2009). In healthy corals, up to 90% or more of the daily metabolic requirements are met by the photosynthetically fixed carbon from the Symbiodiniaceae (Muscatine et al. 1981). Therefore, an energy deficit follows bleaching due to the loss of photosynthates from Symbiodiniaceae coupled with usually increased metabolic costs (Rädecker et al. 2021) and altered energy metabolism (Pupier et al. 2024) in response to stress. The energy deficit may be lethal to the coral if the stress does not decrease or if the coral is not recolonised by Symbiodiniaceae (Muller-Parker et al. 2015). In response the corals may employ heterotrophy as a strategy to make up for the energy deficit (Grottoli et al. 2006; Anthony et al. 2009; Houlbrèque and Ferrier-Pagès 2009).

Corals are experiencing a barrage of stressors; however, because of global climate change, thermal stress is considered the primary threat to corals (Ainsworth et al. 2016; Kim et al. 2023). The frequency, intensity, and duration of marine thermal stress events have increased over the last 35 years (Hughes et al. 2018a). Consecutive global mass coral bleaching events were recorded for the first time during 2014-2017 (Skirving et al. 2019). Increased global mass coral bleaching events are predicted for the near future even with conservative global warming predictions (Skirving et al. 2019). Since corals are fundamental in reef functioning, the significant loss of corals has a domino effect detrimentally affecting all coral reef stakeholders by reducing reef habitat complexity which leads to a reduction in productivity in both an ecological and socio-economic sense (Moberg and Folke 1999; Alvarez-Filip et al. 2011; Alvarez-Filip et al. 2013; Dietzel et al. 2021).

Nonetheless, there is large variability in the degree of coral bleaching severity and mortality when corals are faced with thermal stress events. Innate characteristics related to coral morphology, like greater tissue thickness and lipid reserves, as well as lower metabolic rates, are known to contribute to more thermal resilience in massive species compared to branching

species (Coles and Jokiel 1977; Loya et al. 2001; Guest et al. 2012; Levas et al. 2013; Schoepf et al. 2015b; Jacobson et al. 2016). Corals that encounter highly variable thermal regimes, such as in intertidal rock pools, are more resilient to thermal stress than conspecifics that encounter moderate thermal regimes (Smit 2014; Camp et al. 2018a; Safaie et al. 2018; Schoepf et al. 2021; McRae et al. 2022; Huang et al. 2024). However, the underlying physiological mechanisms that drive the variability in coral resilience/susceptibility to thermal stress are still unclear and warrant investigation. Moreover, McLachlan et al. (2020) indicated that two-thirds of heat-stress experiments on corals over three decades (1990-2020) were conducted in only three countries and that three species were more heavily studied than others. Thus, there is still the need to investigate coral performances under thermal stress in corals of differing species and from various locations that experience varying degrees of thermal stress to attain fundamental information that will aid in the conservation of corals.

Therefore, investigating corals from the extreme and marginal habitats of understudied locations such as South Africa, in thermal stress experiments provides an opportunity to better understand the mechanisms behind their resilience or susceptibility to thermal stress. Generally, respiration, photosynthetic, and growth rates are fundamental physiological processes that determine the coral's performance when exposed to thermal stress (Lesser 2013). The Symbiodiniaceae density, Symbiodiniaceae chlorophyll-a concentration, and lipid concentration of corals influence their energy budget, thereby influencing their resilience/susceptibility to stress and are therefore also measured during experiments (Muscatine 1990; Nystrom et al. 2001; Houlbrèque et al. 2003; Rodrigues and Grottoli 2007). The resilience of corals combines their resistance to and recovery from thermal stress, so it is important to also investigate coral performance when thermal stress ceases (Visram and Douglas 2007; Osborne et al. 2017). Therefore, this study aimed to determine the resilience or susceptibility of a massive coral species, *Anomastraea irregularis*, and a branching coral species, *Pocillopora verrucosa*, from environmentally variable intertidal pools and environmentally stable subtidal habitat at Treasure Beach, South Africa, under experimental thermal stress conditions. This was accomplished by measuring the corals' physiological responses i.e., respiration, photosynthetic, and growth rates, as well as change in tissue composition (Symbiodiniaceae density, Symbiodiniaceae chlorophyll-a, and lipid concentrations) over three months of thermal stress and then two months of recovery. This study hypothesised that corals from the environmentally variable intertidal habitat would exhibit greater thermal resilience under the prolonged thermal stress treatments than their

subtidal conspecifics. Furthermore, it was hypothesised that *A. irregularis* would exhibit greater thermal resilience than *P. verrucosa*.

2.3 Materials and methods

2.3.1 Study site

Anomastraea irregularis, a massive species, and *Pocillopora verrucosa*, a branching species, were collected during low tide from the highly variable intertidal and more stable subtidal habitats at Treasure Beach (29°57'32.8"S 30°59'19.0"E), on the east coast of South Africa. The South African coastline is relatively high energy and experiences a semi-diurnal tidal cycle with a tidal range of 1.5-2.23 m (Smit and Glassom 2017). Thus, approximately 4 h twice a day during spring low tides the rock pools at Treasure Beach are isolated and aerially exposed and then completely inundated during hightides (Smit and Glassom 2017). The rock pools from which the corals were sampled were ~0.27 m^2 and ~0.39 m deep and occurred at near-horizontal surfaces between the high- and low-tide marks. The average temperature in the intertidal rock pools in this region is stable however there are large daily fluctuations of up to 10°C over as little as 2 h in summer and during daytime spring low tides (Smit and Glassom 2017). The subtidal zone a few meters beyond the low tide mark was ~3 m deep and considered to be a relatively more moderate thermal habitat since there is no aerial exposure or isolation with tidal changes the daily temperature ranges were believed to be minimal compared to the intertidal habitat. There was no published literature specifically on differences in the levels of turbidity, light, pH, wave action, oxygen, nutrients, etc. experienced in these two habitats. However, based studies from other regions it is highly likely that the light, pH, salinity stress are intensified in the rock pools during low tide isolation (Le Tissier and Brown 1996; Helmuth et al. 2006).

2.3.2 Collection of corals

The two species were chosen as focus species because they are common in the area (Smit and Glassom 2017) and have differing morphology. A hammer and chisel were used to remove ten healthy (colour 6 on Coral Health Chart (Coral Watch, Australia) and with no visual signs of disease or parasites) colonies of each species from the substrata of each habitat. The number of parent colonies was chosen to sustain more than 80% of allelic variability in the coral populations (Baums et al. 2019). Corals were sampled under the South African Department of Environmental Affairs and the Department of Agriculture, Forestry and Fisheries research permit number RES2022-50. No further permission or ethical approval was required (University of KwaZulu-Natal's Animal Research Ethics Committee Standard Operating

Procedures). The colonies were transported to the Marine Science Unit (MSU) at the University of KwaZulu-Natal in plastic buckets filled with seawater from the site. The colonies were left to acclimate for a week in four 160-L recirculating indoor aquaria (the aquaria setup can be seen in **Appendix 2**) filled with sand-filtered natural seawater attained from uShaka Marine World (permit number WSWJ210028). Each tank was equipped with a submersible glass heater (300W, Enjoy Royal, China) to maintain the tank seawater temperature at 26 ± 0.5°C (mean ± standard deviation). This is the long-term annual average temperature at Treasure Beach and was used as the control temperature during this study to reflect typical ambient conditions experienced by corals throughout the year.

2.3.3 Experimental design and maintenance of corals

Each colony was then fragmented into nine pieces (3.1 ± 0.45 cm long for *P. verrucosa* and 3.1 ± 0.41 cm wide for *A. irregularis*) using wire cutters to attain 90 fragments per species from each habitat (**Appendix 1b**). Each coral fragment was stuck onto pre-labelled plastic tiles using epoxy (Pratley® Quickset Putty, South Africa). The coral fragments were then randomly separated into nine 160-L recirculating aquaria (n=10 of each species from each habitat). Therefore, the stocking density for each tank was 40 coral fragments (**Appendix 2**). The coral fragments were left to heal and further acclimate to the indoor aquarium conditions for one month before temperatures in the treatment tanks were increased (**Appendix 1a** shows the full study timeline). The maximum monthly mean (MMM) for the study region is 27°C (NOAA Coral Reef Watch). The two elevated temperatures chosen for this study were 28°C and 30°C. They represented the presumed local bleaching threshold of MMM for the region +1°C and potential worst-case scenario where the local bleaching threshold would be exceeded with an increase of 3°C from the MMM (IPCC 2023). The experimental degree heating weeks based on the temperature loggers in each treatment tank were calculated as per Leggat et al. (2022) and displayed in **Figure 2.1a**.

Following acclimation, three replicate tanks were allocated per treatment (**Appendix 2c**). The three control tanks remained at 26°C while the temperature in the treatment tanks was ramped up at a rate of 0.5°C per day. The temperature in tanks allocated to the 30°C treatment was increased first and took eight days to reach the target temperature, while the temperature in the tanks allocated to the 28°C treatment was increased from day four onwards and reached the target temperature on the same day as the 30°C treatment. The elevated temperature conditions in the treatment tanks were maintained for approximately three months after which the temperatures were ramped down to the control temperature at 0.5°C per day by reducing the

temperature on the submersible glass heaters. All nine tanks were then maintained at an average temperature of 26°C for approximately two months.

A submersible pump with an output of 4000 L h^{-1} in each sump maintained constant water flow in each tank throughout the acclimation and experimental study period. Each tank was illuminated daily for 12 hours with an artificial LED (light emitting diode) aquarium light (Zetlight 6600 qmavenII, China) throughout the acclimation and experimental study period. To prevent sudden overexposure to LED light during the initial acclimation of the colonies, the preprogrammed manufacturer acclimation setting was used. The programme gradually ramped up PAR (photosynthetically active radiation) intensities daily over the course of a week. Afterwards, throughout the study, the light over each tank was programmed to gradually increase from 06:00, reaching a peak midday average of ~245 µmol m^{-2} s^{-1} and then gradually decrease in intensity towards darkness at 18:00. Each tank's average PAR levels were measured using an Apogee PAR meter (MQ-650 ePAR meter, United States of America) and found to range from 100 to 250 µmol m^{-2} s^{-1}. The PAR range represents the spatial variability within each tank at peak midday light levels. The highest intensity was measured at the centre of the tank, while the edges of the tank received lower intensities due to the positioning of the light bulbs because of the design of the light. The physico-chemical seawater parameters were measured regularly in each tank from the start until the end of the study. A temperature logger (iButtons Maxim®, United States of America) was placed in each tank and set to record temperature in 30-minute intervals (**Appendix 3a**). The salinity and pH were measured in each tank daily with a multiprobe water quality meter (P613 Portable combo meter, China) (**Appendix 3b and c**). Distilled water was added to the sumps when needed to maintain salinity at approximately 35 g kg^{-1}. The alkalinity, calcium, and magnesium levels in each tank were measured weekly using commercial kits (Red Sea, Reef Foundation Pro Multi Test Kit, Israel). Alkalinity was maintained at approximately 1428.8 µmol kg^{-1}, calcium was maintained at approximately 420 mg kg^{-1}, and magnesium was maintained at approximately 1280 mg kg^{-1} by dosing with commercial liquid supplements when necessary, following the manufacturer's instructions (Red Sea, Reef Foundation A, B, C+ Complete Pack, Israel). The accuracy of the test kits was: ± 25 µmol kg^{-1} for alkalinity, ± 5 mg kg^{-1} for calcium and ± 20 mg kg^{-1} for magnesium (Red Sea, Reef Foundation A, B, C+ Complete Pack, Israel). Every week, seawater changes of approximately 50% fresh sand-filtered natural seawater, cotton filter changes were performed, and the tanks were cleaned of debris and algae. The coral fragments were also rotated within the tanks weekly to minimise potential positional effects of light and/or water

circulation. Four mL of coral nutrition (Red Sea, Reef Energy Plus (AB+), Israel) was also added to each tank weekly.

2.3.4 Determination of physiological rates

The health of each coral fragment was monitored visually using the Coral Health Chart (Siebeck et al. 2006) approximately every week (23 sampling occasions in total see **Appendix 1c** for exact sampling points) from the beginning of ramping up temperatures in the treatment tanks, start of the heat stress (when the treatment tanks reached the desired 28°C and 30°) exposure until the end of the study. The number of fragments that died was noted for each tank, and the overall percentage mortality was calculated for each treatment on each occasion.

The photosynthetic and respiration rates were measured on seven different occasions (see **Appendix 1c** for exact sampling points) from before the start of thermal stress (23 July 2022) and then approximately monthly until the end of the study (20 December 2022). Three fragments of each species and from each habitat were randomly selected from each tank, and each fragment was placed in individual sealable 2-L glass jars filled with natural seawater and equipped with a submersible pump (385 L h^{-1}, Dophin, China) to ensure constant water movement during the three-hour incubations (Boodraj and Glassom 2022), first under LED light and then under dark conditions. The incubation jars were kept in water baths maintained at the tank temperatures from which the fragments originated. Three experimental blank control chambers filled with natural seawater and a submersible pump but with no coral fragment were used as blank controls for each temperature treatment to correct for any non-coral metabolic activity within the seawater. Detailed methods in chapter 4 describe the preliminary trials and the calculation of respiration and photosynthesis from the dissolved oxygen measurements. The surface area of each fragment was used to normalise the respiration and photosynthetic rates and was attained using the aluminium foil wrap method (Marsh 1970) at the end of the experiment.

The buoyant weight method (Davies 1989) was used to measure the skeletal growth rate of each coral fragment. Each fragment was weighed on seven different occasions (see **Appendix 1c** for exact sampling points) from before the start of thermal stress (23 July 2022) and then approximately monthly until the end of the study (20 December 2022). An analytical mass balance (Radwag AS 220.R2 PLUS, Germany, accuracy ± 0.1 mg) with an under-weighing capability was used with a monofilament line with a fishing hook attached to the end. The fragments were weighed in a jar of seawater from their respective tanks to reduce the stress of

environmental changes. For the density calculations, a metal 50-g calibration weight was weighed to determine the seawater density and the density of aragonite (2.94 g cm^{-3}) was used for the coral fragments (Davies 1989). The change in mass (mg) between times for each fragment was normalised to surface area and number of days between measurements to attain the growth rate in mg cm^{-2} day^{-1}.

2.3.5 Determination of tissue composition

At the start (31 July 2022) and end (23 October 2022) of the thermal stress and at the end of the recovery period (20 December 2022) three fragments of each species from each habitat from the control and treatment groups were removed. The fragments were flash-frozen in liquid nitrogen and kept in a -80°C ultra-freezer (Snijders Labs, VF475-86, Germany) until further processing. The coral tissue from the fragments was then removed using an Aquaflosser filled with autoclaved seawater (Johannes and Wiebe 1970). The coral tissue was diluted to a standard volume with autoclaved seawater and homogenized in a beaker using a homogeniser (Ultra-Turrax®IKA® T25 digital, Germany). Each sample homogenate was divided into three parts to determine Symbiodiniaceae cell densities, Symbiodiniaceae chlorophyll-a concentration, and lastly, total lipid content (Boodraj 2019; Boodraj and Glassom 2022).

The Symbiodiniaceae cell density was counted and normalised as described by Boodraj and Glassom (2022) but there were amendments to the chlorophyll-a concentration and lipid content analyses. The 15 mL of coral holobiont tissue homogenate was centrifuged at 1200 x g for ten minutes at 4°C to separate the Symbiodiniaceae cells from the coral tissue (Gamba et al. 2022). Two mL of the supernatant containing the coral tissue was pipetted into Eppendorf tubes and immediately stored in a -80°C-ultra-freezer to be used later for biochemical biomarker analyses described in Chapter 3. The pellet containing the algal cells were then treated and analysed as described by Boodraj and Glassom (2022). The Symbiodiniaceae efficiency of the fragments was determined by dividing the chlorophyll-a concentration by the Symbiodiniaceae densities (Boodraj and Glassom 2022). The amendments to Boodraj and Glassom (2022) method to determine the lipid content were that the tissue homogenate was centrifuged at 1200 x g for ten minutes and that the pellet was resuspended in six mL of 2:1 (v/v) chloroform:methanol (Miktek, South Africa). Thereafter the lipid content determination and normalisation followed Boodraj and Glassom (2022).

2.3.6 Statistical analysis

All the statistical analyses performed in the thesis were done using the statistical programme IBM SPSS version 28. To determine if there were significant differences in coral health score, respiration rates, photosynthetic rates, P:R ratios, and growth rates between habitats (intertidal and subtidal) and temperature treatments (control (26°C), 28°C, and 30°C) at the different times (see **Appendix 1c** for exact times for each variable) for each species five Generalised Estimating Equations (GEE) were performed. The residuals of the dependent variables were found to be nonparametric (one sample Kolmogorov-Smirnov $p < 0.05$) therefore GEEs were performed with a gamma distribution with log link functions. The colony effect and tank replicate were added as covariates to account for random effects but were removed from the models if they were non-significant. The GEEs with time effect does account for repeated measures, as they are specifically designed to account for repeated measures and correlated data in longitudinal or clustered data settings when the assumption of normality is not met (Hubbard et al. 2010).

To determine if there were significant differences in Symbiodiniaceae cell density, Symbiodiniaceae chlorophyll-a concentration, chlorophyll-a per Symbiodiniaceae cell, and lipid concentration between habitats (intertidal and subtidal) and temperature treatments (control (26°C), 28°C, and 30°C) at the different times (see **Appendix 1c** for exact times for each variable) four Generalised Linear Models (GLM) were performed for each species. GLMs were performed with a gamma distribution with log link functions as the residuals were nonparametric (one sample Kolmogorov-Smirnov on residuals of the dependent variables $p < 0.05$). The colony effect and tank replicate were added as covariates to account for random effects but were removed from the models if they were non-significant. To determine statistical differences within tested groups Bonferroni pairwise comparisons using estimated marginal means were also performed.

2.4 Results

2.4.1 Bleaching and mortality

Coral fragments of both species from both habitats in the control tanks remained healthy, with no colour loss or mortality recorded throughout the study (**Figure 2.1b** and **2.2**). The average DHW remained more pronounced in the 30°C treatment (overall mean 21.3 ± 4.17 standard deviation) than the 28°C treatment (overall mean 7.71 ± 2.29 standard deviation) throughout the study (**Figure 2.1a**). The coral score of both species in the two elevated temperature treatments significantly differed from their conspecifics in the control throughout the study

(**Table 2.1**, **Figure 2.1b**). Bonferroni pairwise analysis shows exactly when the coral scores significantly differed with the mean differences between pairs for each species (**Appendix 4**). Overall, subtidal and intertidal *P. verrucosa* fragments, and subtidal *A. irregularis* fragments in both temperature treatments began to lose colour after approximately a week of thermal stress (**Figure 2.1b**). In contrast, colour loss in intertidal *A. irregularis* in both temperature treatments was only seen after approximately a month of thermal stress (**Figure 2.1b**). Overall, after three months of thermal stress, the fragments of both species from both habitats experienced significant bleaching, with those in the 30°C treatment experiencing more bleaching than those in the 28°C (**Figure 2.1b**, **Table 2.1**, **Appendix 4**). Overall, *A. irregularis* in the 30°C and 28°C had ~31% and ~25% lower coral scores compared to the control, while *P. verrucosa* had ~40% and~34% lower coral scores than the control. In general, the subtidal fragments of both species experienced more colour loss than their intertidal counterparts (**Figure 2.1b**, **Appendix 4**). Overall, subtidal *A. irregularis* had ~13% and subtidal *P. verrucosa* had ~7% lower coral scores than their respective intertidal conspecifics (**Figure 2.1b**, **Appendix 4**). At the end of two months of recovery, there was improvement in colour in the fragments of both species from both habitats, although the scores remained lower than the initial values (**Figure 2.1b, Appendix 4**). Overall, subtidal *A. irregularis* had ~21% and intertidal *A. irregularis* had ~17% lower coral scores at the end of recovery than the initial scores while subtidal *P. verrucosa* had ~28% and intertidal *P. verrucosa* had ~25% lower coral scores at the end of recovery (**Figure 2.1b**).

Mortality mostly coincided with the bleaching trend (**Figure 2.1b** and **2.2**). Overall, *P. verrucosa* experienced ~300% higher mortality than *A. irregularis* in the 30°C treatment and ~900% higher in the 28°C treatment (**Figure 2.2**). Furthermore, no *A. irregularis* intertidal fragments died during the study, while *P. verrucosa* subtidal fragments in the 30°C treatment experienced ~220% and ~67% higher mortality than their intertidal fragments in the same treatments (**Figure 2.2**). After approximately two months of thermal stress, no intertidal fragments died but subtidal fragments experienced mortalities, with ~100% higher deaths recorded for *P. verrucosa* in the 30°C treatment than in the 28°C treatment and only ~4% mortality recorded for *A. irregularis* in the 30°C treatment and no deaths in *A. irregularis* in the 28°C (**Figure 2.2**). *Pocillopora verrucosa* fragments from the intertidal habitat only experienced mortalities after three months of thermal stress, and ~20% higher deaths were recorded in the 28°C treatment than in the 30°C treatment (**Figure 2.2**). After the first month of recovery one subtidal *A. irregularis* fragment from the 30°C treatment, two subtidal *P.*

verrucosa fragments from the 30°C and one subtidal *P. verrucosa* from the 28°C treatment died (**Figure 2.2**). No other mortalities were recorded at the end of the second month of recovery (**Figure 2.2**).

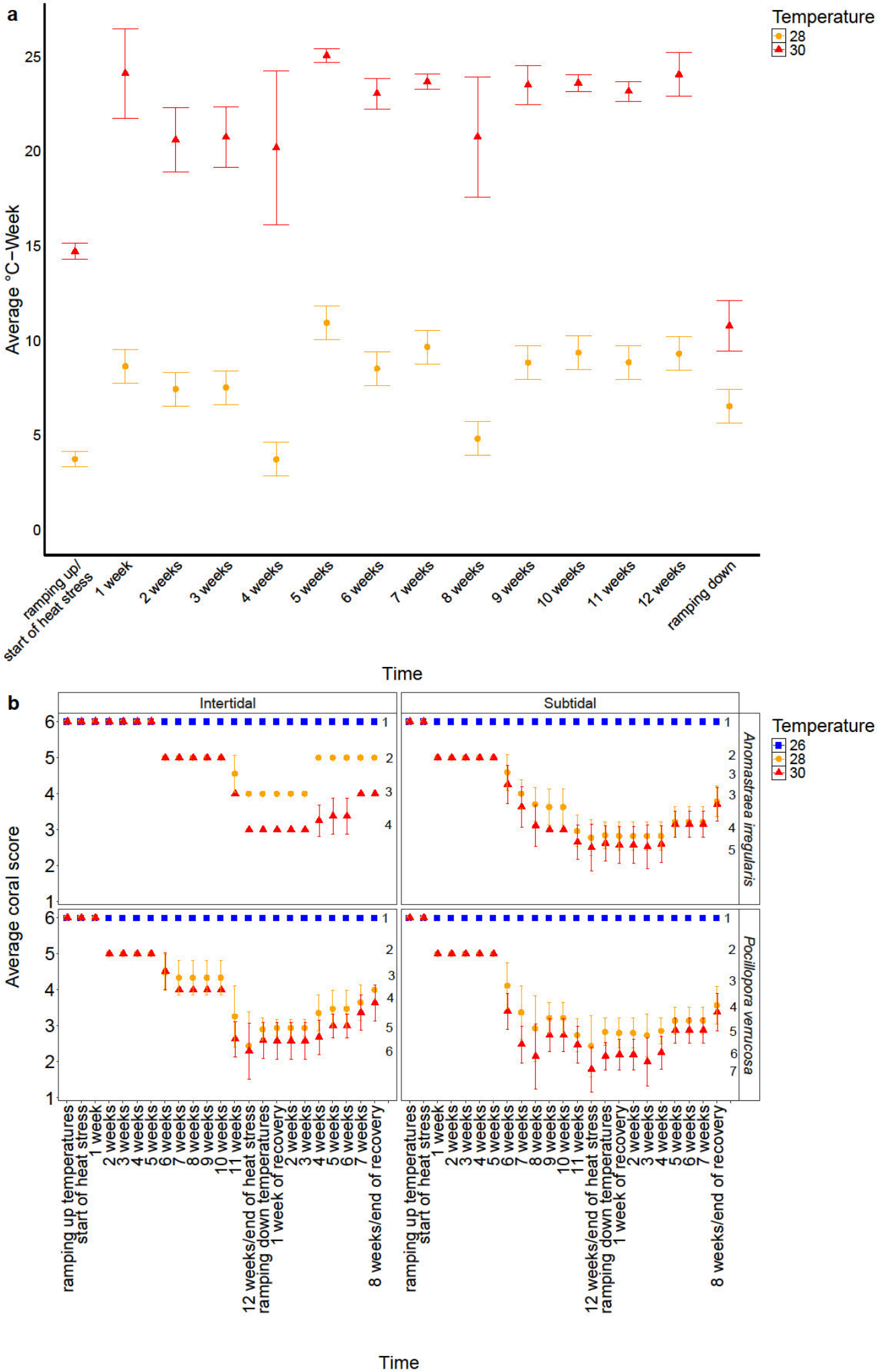
a
Temperature
28
30
Average °C−Week
0
5
10
15
20
25
ramping up/
start of heat stress
1 week
2 weeks
3 weeks
4 weeks
5 weeks
6 weeks
7 weeks
8 weeks
9 weeks
10 weeks
11 weeks
12 weeks
ramping down
Time
b
Intertidal
Subtidal
Anomastraea irregularis
Pocillopora verrucosa
Temperature
26
28
30
Average coral score
ramping up temperatures
start of heat stress
1 week
2 weeks
3 weeks
4 weeks
5 weeks
6 weeks
7 weeks
8 weeks
9 weeks
10 weeks
11 weeks
12 weeks/end of heat stress
ramping down temperatures
1 week of recovery
2 weeks
3 weeks
4 weeks
5 weeks
6 weeks
7 weeks
8 weeks/end of recovery
Time

Figure 2.1: **a** The average experimental degree heating weeks of the treatments ± 1 standard deviation and **b** Average coral score ± 1 standard deviation of *Anomastraea irregularis* and *Pocillopora verrucosa* fragments from the intertidal and subtidal habitats when exposed to the control (26°C) and two thermal stress treatments (28°C and 30°C) over the experimental period. Numbers (1-7) indicate significant differences according to Bonferroni post-hoc tests for GEEs for each species.

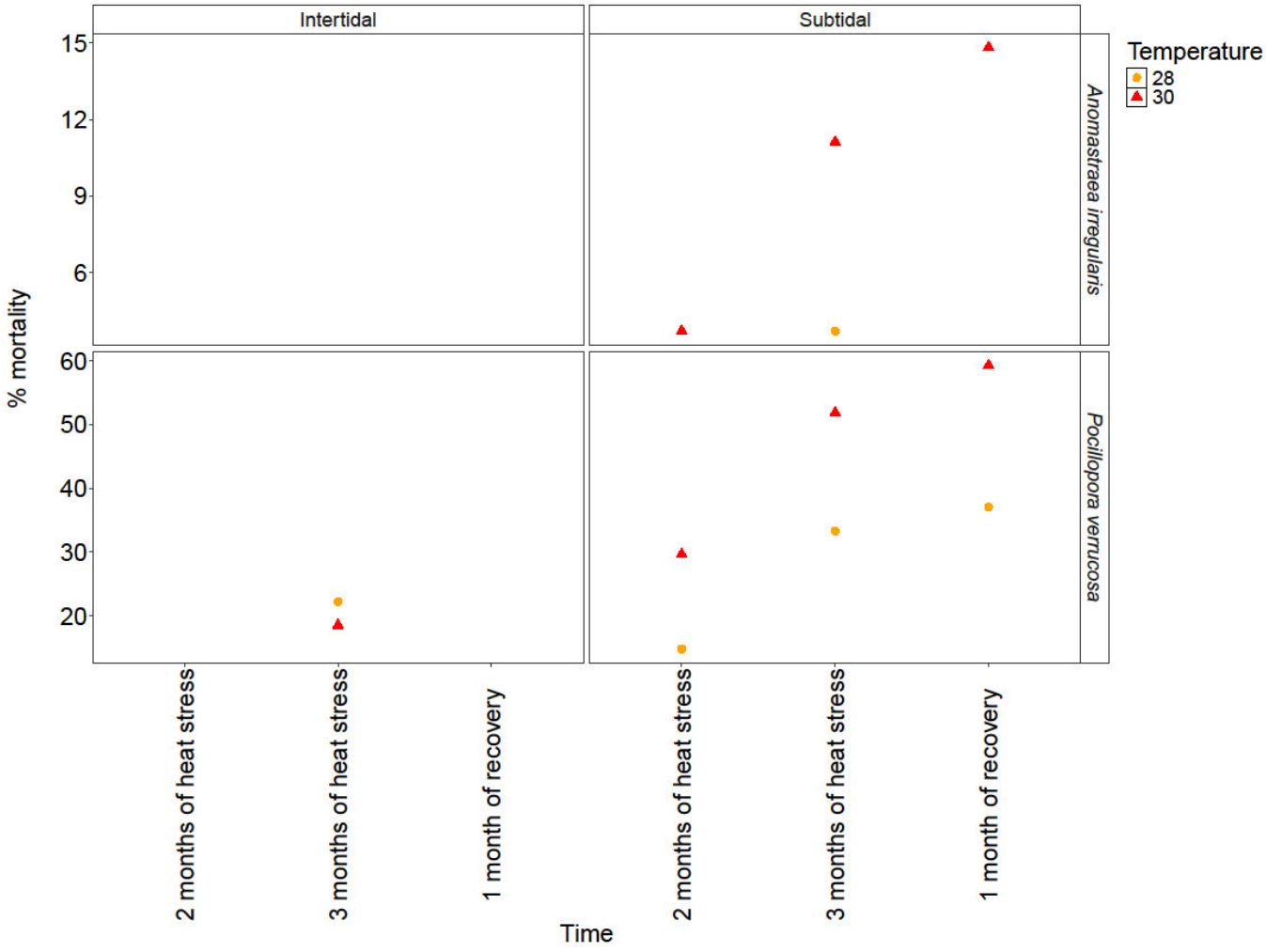

Figure 2.2: The cumulative mortality (%) of *Anomastraea irregularis* and *Pocillopora verrucosa* fragments from the intertidal and subtidal habitats during the study when exposed to the two thermal stress treatments (28°C and 30°C)

Table 2.1: GEE results for each species comparing the coral score, metabolic rates, and growth rates between habitat (intertidal and subtidal), treatments (control, 28°C, and 30°C), times.

		Anomastraea irregularis			*Pocillopora verrucosa*		
Physiological variable	Factor	Wald Chi-Square	df	*p*	Wald Chi-Square	df	*p*
Coral score	Time	36363966501	22	<0.0005	1899704.995	22	<0.0005
	Treatment	1328.272	2	<0.0005	10168.347	2	<0.0005
	Habitat	63.866	1	<0.0005	122.425	1	<0.0005
	Time x treatment x habitat	566.978	26	<0.0005	14012054.898	26	<0.0005
Respiration rate	Time	91.379	6	<0.0005	1437.253	6	<0.0005
	Treatment	583.92	2	<0.0005	10171.758	2	<0.0005
	Habitat	3073.423	1	<0.0005	21334.439	1	<0.0005
	Time x treatment x habitat	19.936	10	0.03	473.485	10	<0.0005
Gross photosynthesis rate	Time	42.397	6	<0.0005	699.891	6	<0.0005
	Treatment	1741.28	2	<0.0005	12698.579	2	<0.0005
	Habitat	3121.952	1	<0.0005	23788.924	1	<0.0005
	Time x treatment x habitat	26.132	10	<0.0005	250.186	10	<0.0005
P:R	Time	52.013	6	<0.0005	834.055	6	<0.0005
	Treatment	1830.383	2	<0.0005	9576.727	2	<0.0005
	Habitat	3601.191	1	<0.0005	21064.934	1	<0.0005
	Time x treatment x habitat	23.562	10	0.009	166.546	10	<0.0005
Growth rate	Time	144280.289	5	<0.0005	169142.083	5	<0.0005
	Treatment	246116.28	2	<0.0005	356853.508	2	<0.0005
	Habitat	570352.695	1	<0.0005	218971.667	1	<0.0005
	Time x treatment x habitat	52184.223	10	<0.0005	104444.508	10	<0.0005

2.4.2 Respiration and photosynthetic rates

The respiration, photosynthetic rates, and P:R ratios of corals in the control conditions did not significantly differ during the study (**Figure 2.3**). Overall, the respiration rates of both species from both habitats in the thermal stress treatments were significantly higher than those in the control (*A. irregularis*: ~0.7% for 28°C and ~1% for 30°C; *P. verrucosa*: ~7% for 28°C and ~15% for 30°C) (**Figure 2.3a**, **Table 2.1**, **Appendix 5**). Furthermore, the respiration rates of the fragments in the 30°C treatment were higher than the 28°C treatment (*A. irregularis*:

~0.3%; *P. verrucosa*: ~8%) (**Figure 2.3a**, **Table 2.1**, **Appendix 5**). The subtidal fragments of both species had much higher respiration rates than the intertidal fragments overall (*A. irregularis*: ~2%; *P. verrucosa*: ~8%) (**Figure 2.3a**, **Table 2.1**, **Appendix 5**). The respiration rates of the subtidal fragments of both species had a more pronounced difference to controls than the intertidal fragments in both thermal stress treatments (subtidal *A. irregularis*: ~0.7% higher for 28°C compared to 26°C and ~1% for 30°C while intertidal *A. irregularis*: ~0.3% for 28°C and ~0.6% for 30°C; subtidal *P. verrucosa*: ~7% for 28°C and ~15% for 30°C while intertidal *P. verrucosa*: ~1% for 28°C and ~3% for 30°C) (**Figure 2.3a**, **Table 2.1**, **Appendix 5**). The respiration rates of both species in the thermal stress treatments began to decrease towards the end of the thermal stress and recovery period (subtidal *A. irregularis*: ~0.5% lower for 28°C at end of recovery compared to end of thermal stress and ~0.4% for 30°C while intertidal *A. irregularis*: ~0.3% for 28°C and ~0.4% for 30°C; subtidal *P. verrucosa*: ~2% for 28°C and ~4% for 30°C while intertidal *P. verrucosa*: ~0.2% for 28°C and ~0.4% for 30°C) (**Figure 2.3a**, **Appendix 5**). However, at the end of the recovery period, the respiration rates of the subtidal fragments of both species in the thermal stress treatments were still significantly higher than the controls (subtidal *A. irregularis*: ~0.4% higher for 28°C compared to 26°C and ~0.5% for 30°C; subtidal *P. verrucosa*: ~4% for 28°C and ~10% for 30°C) (**Figure 2.3a**, **Appendix 5**). At the end of the recovery period, the respiration rates of the intertidal fragments of both species in the thermal stress treatments were similar to their conspecifics in the control (**Figure 2.3a**, **Appendix 5**). Throughout the study *A. irregularis* fragments from both habitats and in all treatments appeared to have higher respiration rates than *P. verrucosa* fragments (**Figure 2.3a**). Overall *A. irregularis* had ~160% higher respiration rates than *P. verrucosa*.

In general, the intertidal fragments of both species had higher gross photosynthetic rates than the subtidal fragments (*A. irregularis*: ~8%; *P. verrucosa*: ~9%) (**Figure 2.3b**, **Table 2.1**, **Appendix 5**). Overall, the gross photosynthetic rates of both species of both habitats in the thermal stress treatments were significantly lower than those in the control, with those in the 30°C treatment being lower than the 28°C treatment (*A. irregularis*: ~7% for 28°C and ~8% for 30°C; *P. verrucosa*: ~3% for 28°C and ~8% for 30°C) (**Figure 2.3b**, **Table 2.1**, **Appendix 5**). During the recovery period, there was a slight increase in the gross photosynthetic rates of the fragments in both thermal stress treatments, more especially in the subtidal fragments although not to rates of the control fragments (subtidal *A. irregularis*: ~2% higher for 28°C at end of recovery compared to end of thermal stress and ~2% for 30°C while intertidal *A. irregularis*: ~2% for 28°C and ~2% for 30°C; subtidal *P. verrucosa*: ~2% for 28°C and ~3%

for 30°C while intertidal *P. verrucosa*: ~0.3% for 28°C and ~1% for 30°C) (**Figure 2.3b**, **Table 2.1**, **Appendix 5**). The decrease in gross photosynthetic rates was more pronounced in thermally stressed intertidal *A. irregularis* fragments as compared to their thermally stressed subtidal conspecifics while the decrease was more pronounced in the thermally stressed subtidal *P. verrucosa* fragments as compared to their thermally stressed intertidal conspecifics (subtidal *A. irregularis*: ~5% lower for 28°C compared to 26°C and ~6% for 30°C while intertidal *A. irregularis*: ~9% for 28°C and ~11% for 30°C; subtidal *P. verrucosa*: ~5% for 28°C and ~7% for 30°C while intertidal *P. verrucosa*: ~2% for 28°C and ~4% for 30°C) (**Figure 2.3b**, **Table 2.1**, **Appendix 5**). Throughout the study, *A. irregularis* fragments from both habitats and all treatments had lower gross photosynthetic rates than *P. verrucosa* fragments from both habitats (**Figure 2.3b**). Overall *A. irregularis* had ~40% lower respiration rates than *P. verrucosa.*

Overall, the fragments of both species from both habitats in the thermal stress treatments had significantly lower P:R ratios than those in the control (*A. irregularis*: ~7% for 28°C and ~9% for 30°C; *P. verrucosa*: ~6% for 28°C and ~14% for 30°C) (**Figure 2.3c**, **Table 2.1**, **Appendix 5**). Despite some recovery of the respiration rates, the lower photosynthetic rates resulted in lower P:R ratios for the thermally stressed corals even after the recovery period (subtidal *A. irregularis*: ~4% lower for 28°C compared to 26°C at the end of recovery and ~4% for 30°C while intertidal *A. irregularis*: ~8% for 28°C and ~11% for 30°C; subtidal *P. verrucosa*: ~17% for 28°C and ~11% for 30°C while intertidal *P. verrucosa*: ~2% for 28°C and ~3% for 30°C) (**Figure 2.3c**, **Table 2.1**, **Appendix 5**). Although the *P. verrucosa* fragments from both habitats were significantly lower when thermally stressed, the ratios were still above 1, while the ratios of *A. irregularis* fragments from both habitats were below 1 even in the controls (**Figure 2.3c**, **Table 2.1**, **Appendix 5**).

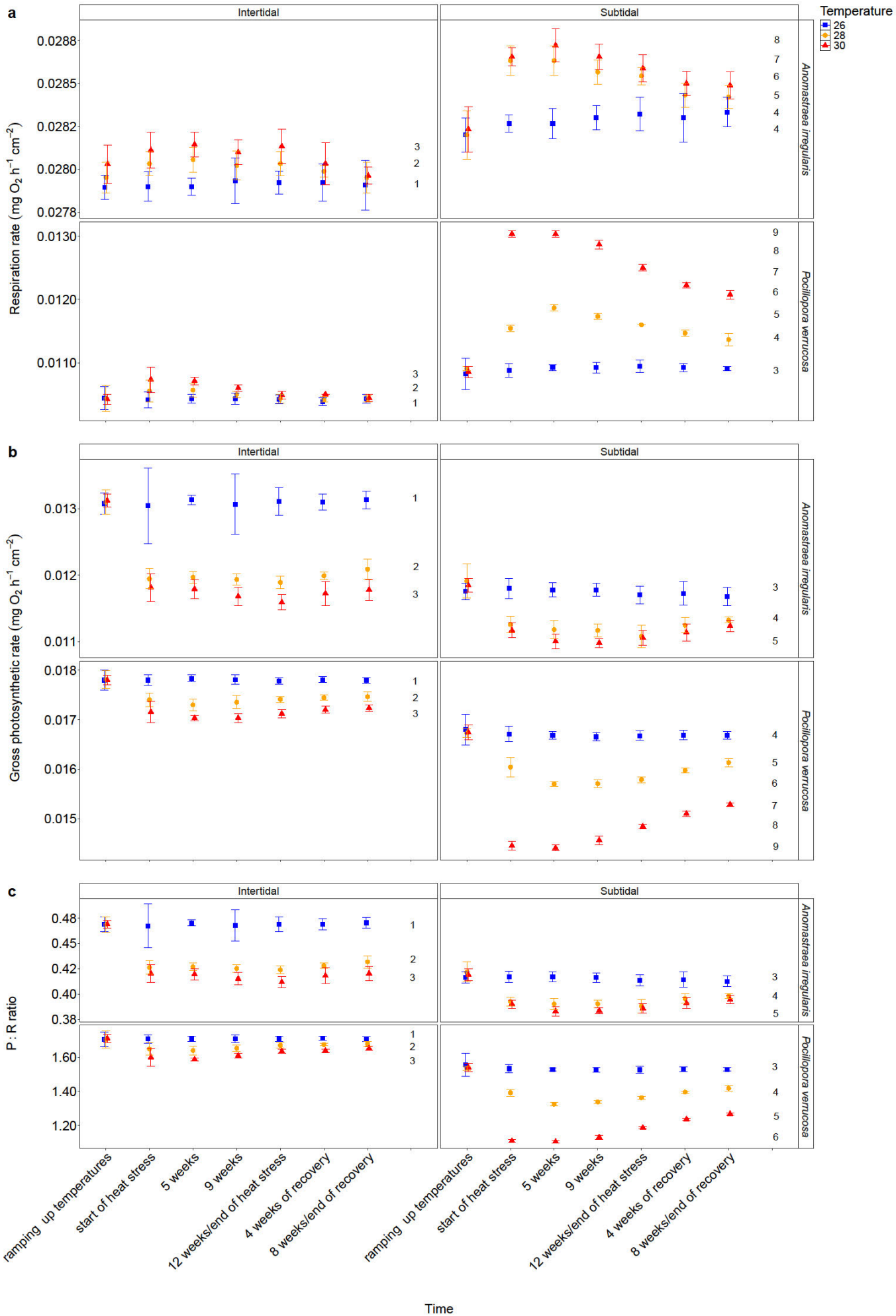
a
b
c
Intertidal
Subtidal
Temperature
26
28
30
Anomastraea irregularis
Pocillopora verrucosa
Respiration rate (mg O2 h-1 cm-2)
Gross photosynthetic rate (mg O2 h-1 cm-2)
P : R ratio
0.0288
0.0285
0.0282
0.0280
0.0278
0.0130
0.0120
0.0110
0.013
0.012
0.011
0.018
0.017
0.016
0.015
0.48
0.45
0.42
0.40
0.38
1.60
1.40
1.20
ramping up temperatures
start of heat stress
5 weeks
9 weeks
12 weeks/end of heat stress
4 weeks of recovery
8 weeks/end of recovery
Time

Figure 2.3: **a** Average respiration rate (mg O_2 h^{-1} cm^{-2}) ± 1 standard deviation, **b** Average gross photosynthetic rate (mg O_2 h^{-1} cm^{-2}) ± 1 standard deviation, and **c** Average gross photosynthesis: respiration (P:R) ratio ± 1 standard deviation of *Anomastraea irregularis* and *Pocillopora verrucosa* fragments from the intertidal and subtidal habitats when exposed to the control (26°C) and two thermal stress treatments (28°C and 30°C) over the experimental period. Numbers (1-9) indicate significant differences according to Bonferroni post-hoc tests for GEEs for each species.

2.4.3 Growth rates

There was no significant change in the growth rate of the corals in the control throughout the experiment (**Figure 2.4**). The growth of both species from both habitats exposed to the two thermal stress treatments did not completely cease during the duration of the study (**Figure 2.4**). However, the two thermal stress treatments caused significantly lower growth rates in both species from both habitats than the control fragments, with those in the 30°C treatment having lower growth rates than those in the 28°C treatment (overall *A. irregularis*: ~12% lower for 28°C compared to 26°C and ~18% for 30°C; *P. verrucosa*: ~15% for 28°C and ~24% for 30°C) (**Figure 2.4**, **Table 2.1**, **Appendix 6**). Overall, the growth rates of the subtidal fragments of both species were significantly lower than their intertidal conspecifics, especially when exposed to thermal stress (*A. irregularis*: ~24% lower for 26°C, ~23% for 28°C and ~14% for 30°C; *P. verrucosa*: ~17% lower for 26°C, ~14% for 28°C and ~25% for 30°C) (**Figure 2.4**, **Table 2.1**, **Appendix 6**). During the recovery period, there were no signs of recovery (i.e., a subsequent increase) in the growth rates of any of the thermally treated fragments (subtidal *A. irregularis*: ~26% lower for 28°C compared to 26°C at the end of recovery and ~40% for 30°C while intertidal *A. irregularis*: ~15% for 28°C and ~18% for 30°C; subtidal *P. verrucosa*: ~22% for 28°C and ~38% for 30°C while intertidal *P. verrucosa*: ~18% for 28°C and ~38% for 30°C) (**Figure 2.4**, **Appendix 6**).

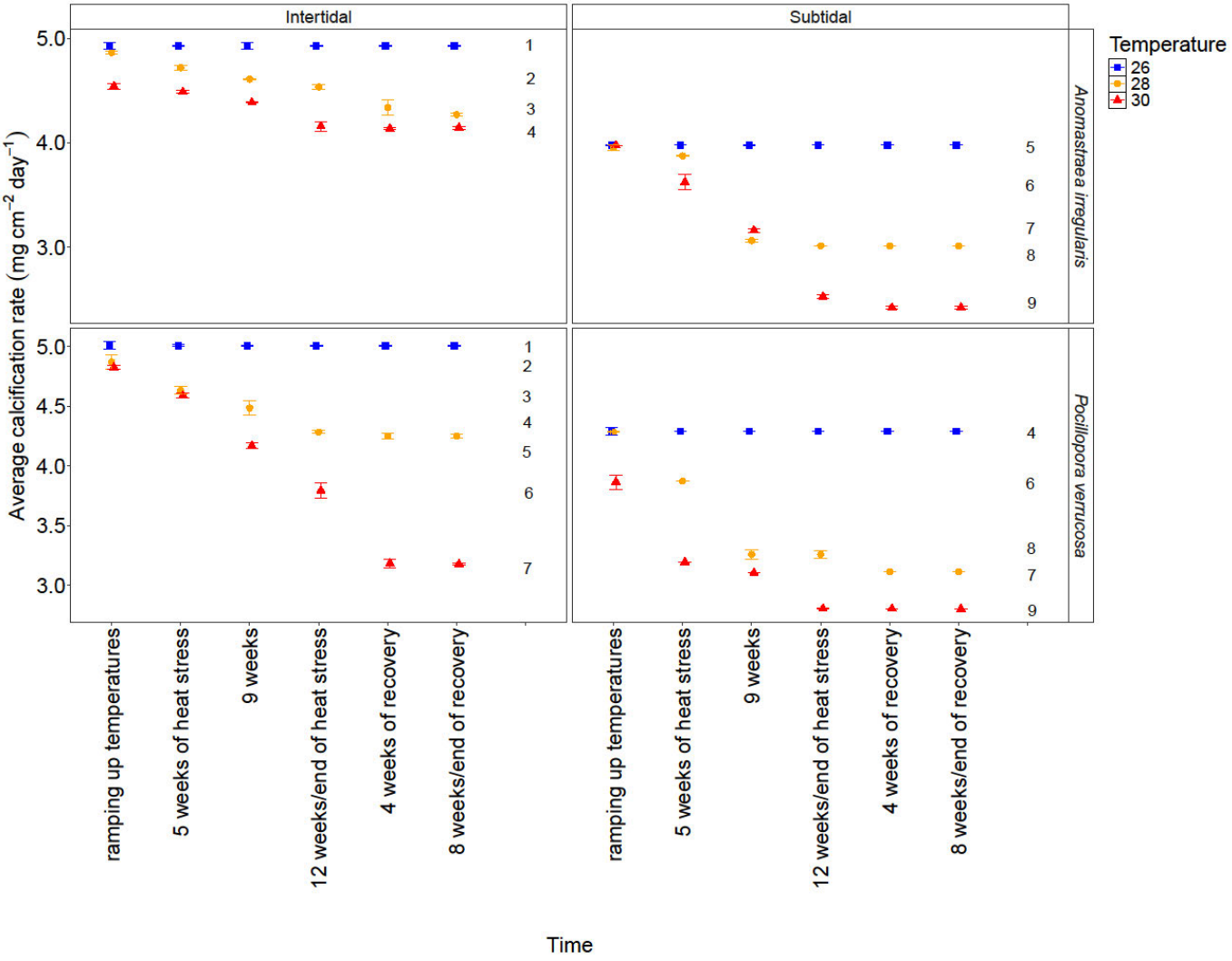

Figure 2.4: Average calcification rate (mg cm^{-2} day^{-1}) ± 1 standard deviation of *Anomastraea irregularis* and *Pocillopora verrucosa* fragments from the intertidal and subtidal habitats after approximately every month from the start until the end of the experiment when exposed to the control (26°C) and two thermal stress treatments (28°C and 30°C) over the course of the study. Numbers (1-9) indicate significant differences according to Bonferroni post-hoc tests for GEEs for each species.

2.4.4 Symbiodiniaceae cell density

Symbiodiniaceae density of the control fragments did not significantly differ throughout the study (**Figure 2.5a**). The two thermal stress treatments caused significantly lower Symbiodiniaceae density in both species from both habitats than the control fragments throughout the study (overall subtidal *A. irregularis* had ~35% lower for 28°C compared to 26°C and ~40% for 30°C while intertidal *A. irregularis* had ~24% for 28°C and ~28% for 30°C; subtidal *P. verrucosa* had ~35% for 28°C and ~44% for 30°C while intertidal *P.*

verrucosa: ~37% for 28°C and ~46% for 30°C) (**Figure 2.5a**, **Table 2.2**, **Appendix 7**). For both species from both habitats, the Symbiodiniaceae density did not significantly differ between the two treatments at the start of the thermal stress, but at the end of the thermal stress and at the end of the recovery period, the Symbiodiniaceae density was significantly lower in the 30°C than those in the 28°C treatment (**Figure 2.5a**, **Table 2.2**, **Appendix 7**). The subtidal *A. irregularis* had ~17% lower cell density at 30°C compared to 28°C at the end of heat stress and ~12% lower at the end of recovery while intertidal *A. irregularis* had ~15% and ~5% lower at the end of heat stress and recovery; subtidal *P. verrucosa* in the 30°C at the end of heat stress was ~24% lower and ~29% lower at the end of recovery while the intertidal *P. verrucosa* was ~30% lower at the end of heat stress and ~29% lower at the end of recovery in the 30°C than those in the 28°C (**Figure 2.5a**, **Table 2.2**, **Appendix 7**). At the end of the recovery period there was an increase in the Symbiodiniaceae density of both species from both habitats, but still not to the control levels (subtidal *A. irregularis*: ~42% lower for 28°C compared to 26°C at the end of recovery and ~49% for 30°C while intertidal *A. irregularis*: ~25% for 28°C and ~29% for 30°C; subtidal *P. verrucosa*: ~48% for 28°C and ~63% for 30°C while intertidal *P. verrucosa*: ~52% for 28°C and ~66% for 30°C) (**Figure 2.5a**, **Table 2.2**, **Appendix 7**). The intertidal fragments of both species had higher Symbiodiniaceae densities than their subtidal conspecifics (**Figure 2.5a**). Overall intertidal *A. irregularis* was ~61% higher than subtidal *A. irregularis* and intertidal *P. verrucosa* was ~12% higher than their subtidal conspecifics (**Figure 2.5a**).

2.4.5 Chlorophyll-a concentration

Chlorophyll-a concentration of the control fragments did not change throughout the study (**Figure 2.5b**). Throughout the study, the two thermal stress treatments caused significantly lower chlorophyll-a concentration in both species from both habitats compared to their conspecifics in the control conditions (**Figure 2.5b**, **Table 2.2**, **Appendix 7**). Overall subtidal *A. irregularis* had ~35% lower for 28°C compared to 26°C and ~41% for 30°C while intertidal *A. irregularis* had ~23% for 28°C and ~28% for 30°C; subtidal *P. verrucosa* had ~35% for 28°C and ~44% for 30°C while intertidal *P. verrucosa*: ~37% for 28°C and ~46% for 30°C (**Figure 2.5b**). For both species from both habitats, the chlorophyll-a concentration did not significantly differ between the two treatments at the start of the thermal stress but at the end of the thermal stress and at the end of the recovery period the chlorophyll-a concentration was significantly lower in the 30°C than those in the 28°C treatment (**Figure 2.5b**, **Table 2.2**, **Appendix 7**). The subtidal *A. irregularis* had ~18% lower cell density at 30°C compared to

28°C at the end of heat stress and ~12% lower at the end of recovery while intertidal *A. irregularis* had ~14% and ~5% lower at the end of heat stress and recovery; subtidal *P. verrucosa* in the 30°C at the end of heat stress was ~24% lower and ~28% lower at the end of recovery while the intertidal *P. verrucosa* was ~30% lower at the end of heat stress and ~29% lower at the end of recovery in the 30°C than those in the 28°C (**Figure 2.5b**, **Table 2.2**, **Appendix 7**). At the end of the recovery period there was an increase in the chlorophyll-a concentration of both species from both habitats, but still not to the control levels (**Figure 2.5b**, **Table 2.2**, **Appendix 7**). Subtidal *A. irregularis*: ~42% lower for 28°C compared to 26°C at the end of recovery and ~49% for 30°C while intertidal *A. irregularis*: ~24% for 28°C and ~28% for 30°C; subtidal *P. verrucosa*: ~48% for 28°C and ~63% for 30°C while intertidal *P. verrucosa*: ~52% for 28°C and ~66% for 30°C (**Figure 2.5b**, **Appendix 7**). Overall intertidal *A. irregularis* was ~49% higher than subtidal *A. irregularis* and intertidal *P. verrucosa* was ~27% higher than their subtidal conspecifics (**Figure 2.5b**).

2.4.6 Chlorophyll-a concentration per Symbiodiniaceae cell

Chlorophyll-a concentration per Symbiodiniaceae cell of the control fragments did not significantly change throughout the study (**Figure 2.5c**). The average chlorophyll-a concentration per Symbiodiniaceae cell in subtidal *A. irregularis* initially exposed to the 30°C thermal stress was ~2% significantly lower than their conspecifics in the control, those in the 28°C treatment only had ~0.7% lower chlorophyll-a concentration per cell than those in the control (**Figure 2.5c**, **Table 2.2**, **Appendix 7**). However, at the end of the thermal stress and recovery period, the average chlorophyll-a concentration per Symbiodiniaceae cell in subtidal *A. irregularis* exposed to the two thermal stress treatments were not significantly different to their conspecifics in the control, nor were there significant differences between the two treatments (**Figure 2.5c**, **Appendix 7**). The average chlorophyll-a concentration per Symbiodiniaceae cell in intertidal *A. irregularis* at the start and end of thermal stress did not significantly differ between the two elevated temperature treatments, nor were they significantly different to their conspecifics in the control (**Figure 2.5c**, **Table 2.2**, **Appendix 7**). Furthermore, at the end of the recovery period the average chlorophyll-a concentration per Symbiodiniaceae cell in intertidal *A. irregularis* in the two thermal treatments was significantly higher than their conspecifics in the control conditions (**Figure 2.5c**, **Table 2.2**, **Appendix 7**). The average chlorophyll-a concentration per Symbiodiniaceae cell in subtidal and intertidal *P. verrucosa* at the start and end of thermal stress as well as at the end of recovery were not significantly different to their conspecifics in the control, nor between the two thermal

treatments (**Figure 2.5c**, **Table 2.2**, **Appendix 7**). Overall, throughout the study, intertidal *P. verrucosa* fragments had ~13% higher chlorophyll-a concentration per Symbiodiniaceae cell than their subtidal conspecifics (**Figure 2.5c**, **Table 2.2**, **Appendix 7**). Conversely, throughout the study, the subtidal *A. irregularis* fragments had ~8% higher chlorophyll-a concentration per Symbiodiniaceae cell than their intertidal conspecifics.

2.4.7 Lipid concentration

Lipid concentration of the control fragments did not significantly change throughout the study (**Figure 2.5d**). At the start of thermal stress, the lipid concentrations of both species from both habitats did not significantly differ between treatments and the control (**Figure 2.5d**, **Appendix 7**). At the end of the thermal stress both species from both habitats had significantly lower lipid concentration when compared to their conspecifics in the control conditions (**Figure 2.5d**, **Table 2.2**, **Appendix 7**). The difference in lipid concentration between the control-28°C and the control-30°C was: ~36% and ~55% for subtidal *A. irregularis*, ~35% and ~42% for intertidal *A. irregularis*, ~9% and ~62% for subtidal *P. verrucosa* and ~32% and ~80% for intertidal *P. verrucosa* (**Figure 2.5d**) At the end of the recovery period, the lipid concentration of both species from both habitats in the two thermal stress treatments did not change from their concentrations at the end of thermal stress (**Figure 2.5d**, **Table 2.2**, **Appendix 7**).For both species from both habitats, the lipid concentration was significantly lower in the 30°C than those in the 28°C treatment at the end of the thermal stress and at the end of the recovery period (**Figure 2.5d**, **Table 2.2**, **Appendix 7**). The subtidal *A. irregularis* had ~31% lower lipid concentration at 30°C compared to 28°C at the end of heat stress while the difference in intertidal *A. irregularis* was ~10%; for subtidal *P. verrucosa* the difference was ~58% and ~71% for intertidal *P. verrucosa* (**Figure 2.5d**). Overall, the intertidal fragments of both species had higher lipid concentrations than their subtidal conspecifics (~21% difference for *A. irregularis* and ~36% difference for *P. verrucosa*) (**Figure 2.5d**, **Appendix 7**). However, the lipid concentrations of the intertidal and subtidal *P. verrucosa* in the 30°C treatment did not significantly differ at the end of thermal stress and end of recovery (**Figure 2.5d**, **Appendix 7**).

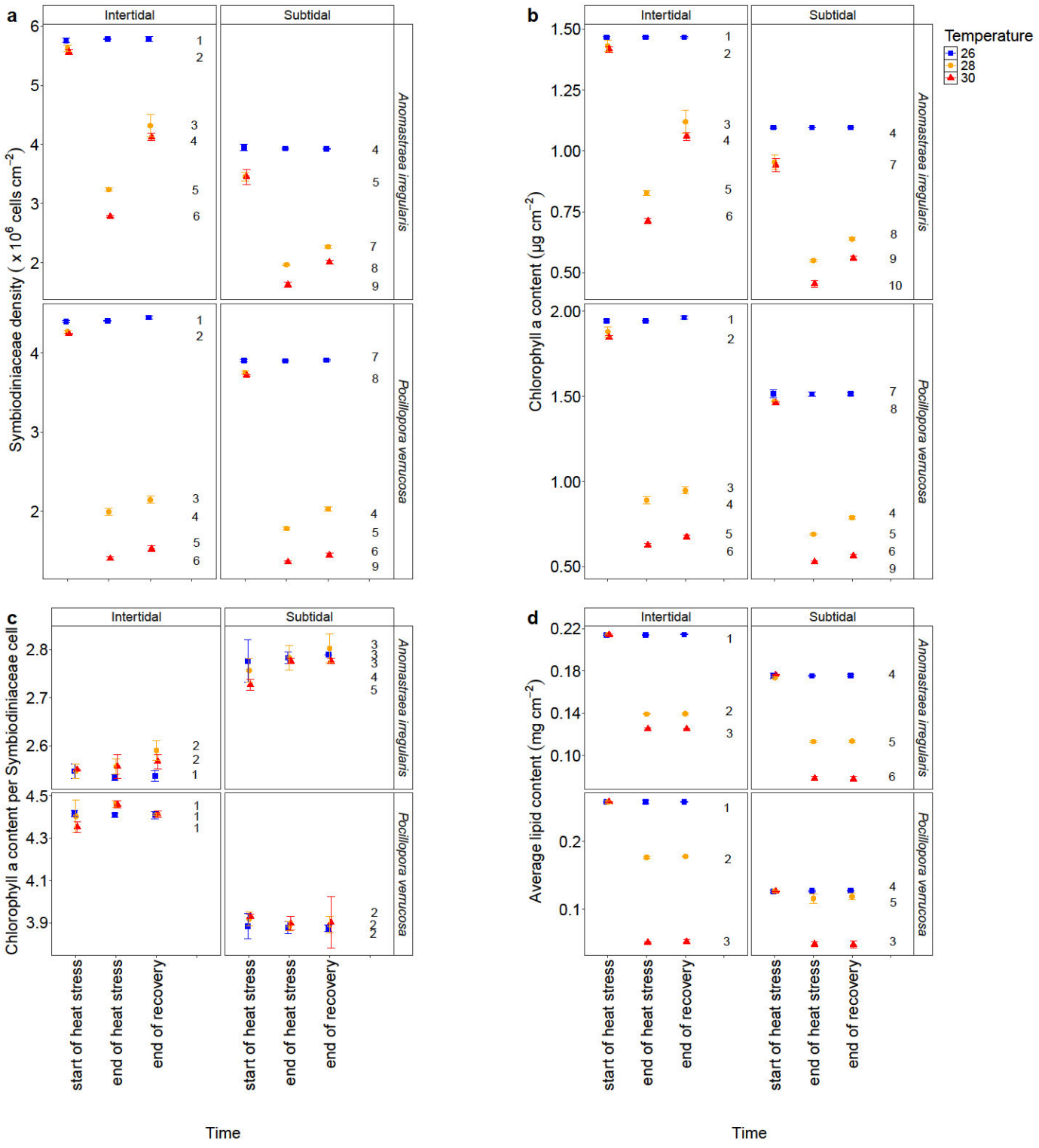

Figure 2.5: **a** Average Symbiodiniaceae cell density (number of cells. cm^{-2}) ± 1 standard deviation, **b** average chlorophyll-a concentration (µg. cm^{-2}) ± 1 standard deviation, **c** average chlorophyll per Symbiodiniaceae cell ± 1 standard deviation, and **d** average lipid concentration (mg. cm^{-2}) ± 1 standard deviation in *Anomastraea irregularis* and *Pocillopora verrucosa* fragments from the intertidal and subtidal habitats at the start and end of thermal stress and at

the end of recovery. Numbers (1-10) indicate significant differences according to Bonferroni post-hoc tests for each species.

Table 2.2: GLM results for each species comparing the Symbiodiniaceae cell density, Symbiodiniaceae chlorophyll-a concentration, and chlorophyll-a per Symbiodiniaceae cell ratio between habitat (intertidal and subtidal), treatments (26°C, 28°C, and 30°C), and times (at the start and end of the experimental thermal stress and end of recovery)

		Anomastraea irregularis			*Pocillopora verrucosa*		
Variable	Factor	Wald Chi-Square	df	*p*	Wald Chi-Square	df	*p*
Symbiodiniaceae cell density	Time	6405.425	2	<0.0005	75347.438	2	<0.0005
	Treatment	9376.042	2	<0.0005	96396.753	2	<0.0005
	Habitat	15599.463	1	<0.0005	4127.002	1	<0.0005
	Time x treatment x habitat	201.221	4	<0.0005	380.217	4	<0.0005
Chlorophyll a concentration	Time	6023.104	2	<0.0005	40841.888	2	<0.0005
	Treatment	9208.626	2	<0.0005	52047.739	2	<0.0005
	Habitat	10473.748	1	<0.0005	10054.645	1	<0.0005
	Time x treatment x habitat	154.231	4	<0.0005	427.722	4	<0.0005
Chlorophyll a concentration per Symbiodiniaceae cell	Time	25.851	2	<0.0005	2.225	2	0.329
	Treatment	8.075	2	0.018	2.453	2	0.293
	Habitat	2659.769	1	<0.0005	3136.810	1	<0.0005
	Time x treatment x habitat	2.104	4	0.043	6.899	4	0.141
Lipid content	Time	8689.889	2	<0.0005	38360.761	2	<0.0005
	Treatment	15805.412	2	<0.0005	41287.548	2	<0.0005
	Habitat	19081.889	1	<0.0005	21487.548	1	<0.0005
	Time x treatment x habitat	1534.527	4	<0.0005	318.030	4	<0.0005

2.5 Discussion

This study measured the physiological responses and recovery of *Anomastraea irregularis* and *Pocillopora verrucosa* from an environmentally variable intertidal habitat and an environmentally stable subtidal habitat in response to long-term experimental thermal stress. The health scores, physiological rates, and tissue content of the control fragments of both

species from both habitats remained fairly unchanged throughout the study (**Figure 2.1b, 2.3, 2.4, and 2.5**) and there were no recorded deaths (**Figure 2.2**) indicating that handling had negligible effect on the corals and that the changes seen in the fragments in the two experimental treatments were in fact due to the thermal stress experienced. This study found that prolonged higher than average temperatures (**Figure 2.1a**) indeed significantly impacted coral physiology and caused bleaching and mortalities. Overall, the results of this study highlighted inherent physiological differences between the species and habitat that were maintained throughout the experiment. The massive species *A. irregularis* appeared more resilient than the branching species *P. verrucosa* in both thermal stress treatments. Literature has shown that branching species can be more susceptible to thermal stress than massive species (Stimson et al. 2002; Bay et al. 2016; Pisapia et al. 2016). In both species, the intertidal corals appeared more resilient than the subtidal corals in both thermal stress treatments. Overall, the results of this study indicate that highly variable habitats and species traits may influence coral resilience to thermal stress.

Adaptive differences can exist between coral populations that experience differential thermal regimes causing thermal resilience in populations that experience highly variable thermal histories (Oliver and Palumbi 2011; Kenkel and Matz 2016; Tkachenko and Soong 2017; Safaie et al. 2018; Tisthammer et al. 2021; Jacquemont et al. 2022; McRae et al. 2022; Speelman et al. 2023). High environmental variability may act as a natural selection pressure, favouring corals with greater thermal tolerance (Oliver and Palumbi 2011; Barshis et al. 2018). Thermally variable habitats can also induce acclimatisation or non-genetic increases in fitness through mechanisms like phenotypic plasticity, symbiosis with stress-tolerant Symbiodiniaceae, maintaining higher levels of heat shock proteins and antioxidants, and epigenetic modifications (Palumbi et al. 2014; Bay and Palumbi 2017; Howells et al. 2020; Thummasan et al. 2021; Lachs et al. 2023). For example, in the Kimberly region of Australia *Acropora aspera* corals exposed to daily temperature variability of up to 7°C developed enhanced bleaching resilience compared to conspecifics from the subtidal habitat where daily temperature variability was moderate (Schoepf et al. 2020; Jung et al. 2021; Schoepf et al. 2021). Similarly, *in situ* temperature data from the intertidal pool at Treasure Beach indicated frequent and high daily fluctuations, with the thermally resilient intertidal corals experiencing daily variability of up to ~5°C compared to highest daily fluctuation of ~3°C in the subtidal habitat (**Figure 5.1a**). This high level of temperature variability may be a forcing factor

influencing the physiological and molecular differences observed between habitats, ultimately influencing their resilience to bleaching.

In both thermal stress treatments, intertidal *A. irregularis* took longer to bleach and experienced less bleaching, and no deaths compared to their subtidal counterparts and subtidal and intertidal *P. verrucosa* (**Figure 2.1b and 2.2**, **Appendix 4**). Intertidal *P. verrucosa* also experienced less bleaching and less deaths than their subtidal counterparts (**Figure 2.1b and 2.2**, **Appendix 4**). This variable thermal resilience/susceptibility may have resulted from the different physiological strategies employed by the two species from the two different habitats. Environmental conditions have important influences on the respiration and photosynthetic performance of scleractinian corals (Ulstrup et al. 2011). Coral respiration rates generally increase or decrease in response to environmental perturbations while no change is indicative of acclimatisation or no stress response (Guppy and Withers 1999; Sawall et al. 2011; Osinga et al. 2012; Al-Sofyani and Floos 2013). Higher respiration rates in response to elevated seawater temperature has been noted for some coral species and is believed to be associated with an increase in the metabolic activity of the corals to counteract the thermal stress (Wooldridge 2014; Sawall et al. 2015; Anderson et al. 2019). The subtidal corals of both species seemed more sensitive to the thermal stress since they maintained higher respiration rates throughout the thermal stress and recovery period when compared to their conspecifics in the control (**Figure 2.3a, Table 2.1, Appendix 5**). Conversely, the intertidal fragments of both species only had higher respiration rates at the start and a month into thermal stress, but by the third month of thermal stress and recovery period their respiration rates were not different to their counterparts in the control (**Figure 2.3a, Table 2.1, Appendix 5**) indicating that they may have acclimatised to the increased temperatures. For both species, there also seemed to be an inherent difference in the respiration rates between habitats. Throughout the study the subtidal corals of both species in both the control and thermal stress treatments had higher respiration rates compared to the intertidal conspecifics (**Figure 2.3a, Table 2.1, Appendix 5**). It is possible that the subtidal corals are not physiologically plastic enough to regulate their respiration rates when faced with above-average temperatures as compared to the intertidal corals that were able to acclimatise to the increased temperatures. This lower physiological plasticity in subtidal corals may be because of the absence of a selective pressure since their moderate habitats do not require large variations in their physiology to survive. Conversely, corals that experience large environmental variability would experience stronger selective pressures that favour corals with greater physiological plasticity to survive the variable

conditions (Ziegler et al. 2014; Schoepf et al. 2015b; Huang et al. 2024). Maintaining high respiration rates is energy-consuming and since bleaching deprives corals of symbiont-derived photosynthates, maintaining the higher respiration rates may have led to more bleaching and higher mortalities of the subtidal corals in the thermal stress treatments (Wooldridge 2014; Anderson et al. 2019; Jacquemont et al. 2022).

Overall, the fragments of both species from both habitats in the thermal stress treatments had significantly lower photosynthetic rates and resultant P:R ratios than those in the control (**Figure 2.3b and c**, **Table 2.1**, **Appendix 5**). Despite the recovery of respiration rates of the intertidal corals and some recovery of the respiration rates of the subtidal corals, the lower photosynthetic rates resulted in significantly lower P:R ratios for the thermally stressed corals even after the recovery period (**Figure 2.3b and c**, **Table 2.1**, **Appendix 5**). Lower P:R ratios can be due to either increased respiration or lower photosynthesis or both (Coles and Jokiel 1977). Reduced P:R ratios are typical in thermally stressed corals due to the significant loss in Symbiodiniaceae cells that follow thermal stress (Coles and Jokiel 1977; Lesser 2013; Muller-Parker et al. 2015). Symbiodiniaceae photosynthesis can generally contribute up to 95% of the daily energy demand of the coral host (Muscatine et al. 1981). Therefore, the proportion of energy the coral host receives from its Symbiodiniaceae can be assessed from the P:R ratio, with a ratio greater than one indicating sufficient energy from the Symbiodiniaceae while a ratio less than one indicates insufficient energy from their Symbiodiniaceae (Muscatine et al. 1981; Baker et al. 2015). Although the thermally stressed *P. verrucosa* fragments from both habitats had significantly lower photosynthetic rates, Symbiodiniaceae density, and chlorophyll-a concentration, the P:R ratios were still above one (**Figure 2.3b and c**, **Table 2.1**). This would imply that despite the thermal stress the Symbiodiniaceae in these fragments were still producing adequate energy for the coral hosts. The chlorophyll-a concentration per Symbiodiniaceae cell of these fragments was not significantly different to the controls at the start and end of the thermal stress (**Figure 2.5c**, **Table 2.2**), which could explain why the P:R ratios remained above one despite the thermal stress. The maintenance or increase in chlorophyll-a per Symbiodiniaceae cell usually occurs with thermally induced bleaching because of the reduced competition for nutrients since there are lower Symbiodiniaceae densities (Jones 1997). It may also be more energetically favourable for the coral host and/or the Symbiodiniaceae to increase the chlorophyll-a content per Symbiodiniaceae cell than to recruit Symbiodiniaceae cells (Rodrigues and Grottoli 2007).

Throughout the study *A. irregularis* fragments from both habitats in the control and thermal stress treatments had higher respiration rates and lower photosynthetic rates compared to *P. verrucosa* (**Figure 2.3a and b**), and the resulting P:R ratios of *A. irregularis* fragments from both habitats were below one even in the controls (**Figure 2.3c**). The higher surface area and thinner tissue of *P. verrucosa* fragments would permit higher photosynthetic rates than the *A. irregularis* fragments which have lower surface area and thicker tissue (Edmunds and Gates 2002; Osinga et al. 2012; Conley and Hollander 2021). Furthermore, through differences in the $\delta^{13}C$ and $\delta^{15}N$ isotopes between coral host tissue and Symbiodiniaceae cells Smit (2014) indicated that heterotrophy contributes more to the metabolism of *A. irregularis* than *P. verrucosa*. Therefore, the difference in P:R ratios between the species could be explained by their heterotrophic/autotrophic dependence to meet their metabolic requirements.

The reduced growth rates of both species from both habitats during long-term thermal stress and recovery from the stress (**Figure 2.4**, **Table 2.1**, **Appendix 6**) is common since growth is an energy consuming process therefore the differential energy shortage following thermal stress prevents the coral hosts from investing energy in growth (Suzuki et al. 2003; Carilli et al. 2009; Colombo-Palotta et al. 2010; Levas et al. 2013; Foster et al. 2014; Razak et al. 2020; Kramer et al. 2022). Although the P:R ratios of *P. verrucosa* indicated adequate energy from the Symbiodiniaceae, the quality of the photosynthates may have not been good enough to allow recovery of growth rates (Tremblay et al. 2016). The lower P:R ratios of the subtidal corals of both species may have caused their lower growth rates as compared to the growth rates of the intertidal corals of both species (**Figure 2.3c and 2.4**). The reduced growth rates of both species from both habitats in response to both thermal stress treatments showed the trade-off for surviving prolonged above-average temperature stress. However, this may not be a universal consequence as there have been instances where corals survived thermal stress without detrimentally impacting their growth rates (Dandan et al. 2015; Wright et al. 2019; Kavousi et al. 2020; Brown et al. 2023).

In general, both species from both habitats had significantly lower Symbiodiniaceae density, chlorophyll-a concentrations, and lipid concentrations at the end of both thermal stress treatments compared to conspecifics in the control (**Figure 2.5a, b, and d**). Thermal stress usually leads to the coral hosts expelling Symbiodiniaceae cells, and it is believed to be a defence mechanism to reduce oxidative stress that leaks out from the algal cells to the coral tissue during stress conditions (Weis 2008; Fujise et al. 2014). The reduction in lipid concentrations could be because the corals were using the lipids as a source of energy

(Rodrigues and Grottoli 2007), or there was a decrease because of damage through lipid peroxidation from the production of reactive oxygen species due to the thermal stress (Lesser 1997; Downs et al. 2002; Onyango 2020). Two months of recovery was not long enough to see significant increases in the Symbiodiniaceae density, chlorophyll-a concentrations, and lipid concentrations in the thermally stressed fragments of both species from both habitats. The time it takes for these properties to completely recover to pre-bleaching values is variable and can take more than eight months in some corals (Rodrigues and Grottoli 2007). This can have implications for coral reefs in the near future because more frequent thermal anomalies may prevent bleached corals *in situ* from recovering from thermal stress events before another event occurs (Hoegh-Guldberg et al. 2023). However, recent studies show that some corals can improve their energy contents after several years of thermal stress, providing some hope for the recovery of bleached corals (Brown et al. 2023; Roik et al. 2023). Overall, the intertidal fragments of both species in the control and thermal stress treatments had higher Symbiodiniaceae densities, chlorophyll-a, and lipid concentrations than their subtidal conspecifics (**Figure 2.5a, b and d**). Usually, corals that have higher Symbiodiniaceae density, chlorophyll-a concentrations, and lipid concentrations are considered to be more resilient to thermal stress (Gates and Edmunds 1999; Stimson et al. 2002; Grottoli et al. 2004; Levas et al. 2013; Grottoli et al. 2017). The intertidal corals may, therefore, be more resilient to thermal stress than their subtidal conspecifics through possessing higher Symbiodiniaceae densities, chlorophyll-a, and lipid concentrations than their subtidal conspecifics even when thermally stressed.

However, there is also evidence from Palau that heat-tolerant corals from warmer patch reefs tended to have lower symbiont densities (Cornwell et al. 2021), and based on the oxidative stress theory of bleaching having higher concentrations of Symbiodiniaceae would put corals at a greater risk of bleaching due to the potentially increased positive feedback loop of reactive oxygen species from the Symbiodiniaceae during thermal stress (Cunning and Baker 2012). Furthermore, there are alternative hypotheses of bleaching indicating that the coral-Symbiodiniaceae may not be mutualistic but in fact a type of farming by the coral that can turn parasitic at times, and that altered carbon and nutrient cycling within the symbiosis during thermal stress leads to bleaching (Wooldridge 2009; Ferrier-Pagès et al. 2018; Morris et al. 2019; Matz 2024). A recent study also found no direct connection between Symbiodiniaceae photodamage and an increase in reaction oxygen species H_2O_2 concentration during thermally induced bleaching (Schlotheuber et al. 2024). Therefore, just having higher or lower

concentrations of Symbiodiniaceae is not enough to induce thermal resilience based on the traditional reactive oxygen bleaching theory and there are definitely more intricate factors at play with the coral-Symbiodiniaceae symbiosis that conveys thermal tolerance. Further energetic and nutrient cycling analyses will be required to decipher the exact role the intertidal coral hosts play in the thermal resilience seen in this study. Furthermore, the Symbiodiniaceae species hosted by the corals in this study was not identified and could have also influenced the corals' resilience/susceptibility to thermal stress. Some coral species were able to acquire increased thermal tolerance by hosting stress resistant Symbiodiniaceae species such as *Durusdinium* spp. and *Cladocopium* spp. (Berkelmans and van Oppen 2006; Stat and Gates 2011; Levas et al. 2013; Hume et al. 2020). Some coral species have been found to shift the relative abundance of their dominant symbionts in response to thermal stress or following thermal stress (Jones et al. 2008; Silverstein et al. 2015; Cunning et al. 2017). Therefore, identifying the Symbiodiniaceae species hosted before, during, and after thermal stress would help in understanding the Symbiodiniaceae's role in these coral's thermal resilience.

Grottoli et al. (2021) recommends feeding corals during long-term bleaching experiments to mimic *in situ* conditions. Therefore, feeding the corals weekly made the study more realistic. However, the seawater used during this study was not filtered enough to remove microscopic organisms, which may have provided additional nutrition, especially to the thermally stressed fragments. The nutritional input from heterotrophy is believed to increase resilience to thermal stress by enabling the coral host to maintain a higher photosynthetic activity and enhance essential nutrients (nitrogen and phosphorous) for the synthesis of antioxidants and heat-stress proteins to counteract harmful reactive oxygen species and their damage to proteins associated with thermal stress (Houlbrèque et al. 2004; Lyndby et al. 2018; Brown et al. 2023). Heterotrophy was not accounted for in this study, and the results could have, therefore, been unintentionally affected by the additional nutrition, especially in *A. irregularis* since they rely more on heterotrophy than *P. verrucosa* (Smit 2014; Boodraj and Glassom 2022). It is also noteworthy that shallow-water corals *in situ* can experience PAR levels which can exceed 500-1000 µmol m^{-2} s^{-1} (Dubinsky et al. 1990; Lehmann 2022). In this study the peak midday PAR was only ~245 µmol m^{-2} s^{-1}, which could have mitigated some of the thermal stress experienced by the corals since reduced light intensity has been found to ameliorate thermal stress effects in some corals (Rosic et al. 2020).

Using controlled laboratory experimental manipulation of coral species to investigate the mechanism of physiological differences in thermal bleaching responses, as conducted in this

study, is important. However, the static nature of these experiments may underestimate the physiological responses of corals to real-world thermal stress since *in situ* corals may face other environmental stressors such as light variations, turbidity, and pollution in addition to thermal stress (Mayfield et al. 2012). Therefore, it is important to also use *in situ* investigations to understand the physiological responses of these corals to thermal stress to better understand them. It will also be important to understand the long-term costs of the physiological mechanisms used to survive the thermal stress in this study. It is believed that the corals' fitness may be impaired as energy resources had to be diverted to the survival mechanisms (Bay and Palumbi 2017). The recovery period of this study also appeared to be too short. Further analysis of the recovery trajectory of these corals is required to understand them better.

Ultimately this study found that both temperature treatments caused bleaching in both species from both habitats, although the 30°C treatment was more detrimental to the corals than the 28°C treatment. This highlights that even the conservatively predicted increase in temperature along the South African east coast can detrimentally affect these corals. In terms of DHW, the corals in this study faced values far exceeding the thresholds of 4 and 8°C-weeks which are believed to cause low-level to severe bleaching (NOAA Coral Reef Watch ; Leggat et al. 2022). Carilli et al. (2012) reported that some coral sites in the Gilbert Islands, Republic of Kiribati experienced DHW values as high as 24°C-weeks and that corals regularly exposed to high and fluctuating temperatures can develop higher thermal tolerances. The degree to which the corals were physiologically affected by the temperature stress varied between species and habitats. Although subtidal corals and *P. verrucosa* in this study exhibited high susceptibility to thermal stress, with more mortalities, those that survived endured up to ~8 and ~21°C-weeks of heat stress by the end of the study. This suggests that while high-latitude corals collectively exhibit notable thermal resilience, there is considerable variation in tolerance across species and habitat, with some individuals demonstrating greater capacity to withstand prolonged thermal stress than others. The results of this study corresponded with McLachlan et al. (2021) proposal that there will be higher survival of corals that can modify their physiology when faced with climate change and local stressors. The intertidal corals of both species and *A. irregularis* appear to have increased resilience to thermal stress over subtidal conspecifics and *P. verrucosa*. Therefore, the results of this study support the hypotheses of this study and highlights that species-specific traits and habitat-driven thermal preconditioning can play critical roles in corals resilience to thermal stress. There seems to be inherent thermal resilience due to the coral's morphology and habitat of origin. The ability to adjust their respiration rates

and maintain higher Symbiodiniaceae density, chlorophyll-a, and lipid concentrations may aid the intertidal corals to survive in their extreme habitats. These characteristics were able to help them persist with the long-term experimental thermal stress in this study. The massive species appeared to be more resilient than the branching species due to its thicker tissue which allowed for higher Symbiodiniaceae density and lipid concentrations. The lower P:R ratios of *A. irregularis*, indicative of more heterotrophic dependence, may have also increased their thermal resilience. Marginal and extreme coral communities have important implications for the conservation and management of coral reefs and therefore, the results of this study are important.

2.6 Conclusion

This study offers insight into the physiological responses of two high-latitude coral species of different morphologies from two habitats of differing thermal regimes when exposed to prolonged experimental thermal stress. The impact of thermal stress varied between species and habitat. This study highlighted that coral resilience to thermal stress can be influenced by habitat of origin and morphology. Corals from the thermally stressful intertidal habitat were more resilient to thermal stress than conspecifics from the subtidal habitat and the massive corals were more resilient than branching corals. The physiological plasticity and maintenance of higher Symbiodiniaceae densities, chlorophyll-a, and lipid concentrations of intertidal corals to persist in their extreme environment is believed to aid in their resilience to long-term thermal stress in this study. The massive species' resilience to long-term thermal stress may have resulted from also having these resilient tissue traits and lower P:R ratios demonstrative of a more heterotrophic nature. The resilience of these corals to long-term experimental thermal stress emphasises the need to study and conserve marginal and extreme coral communities, as they can possess stress-resilient characteristics that are crucial for the conservation of coral reefs in the face of global climate change.

Chapter 3: Antioxidant enzyme activity of intertidal and subtidal coral hosts *Anomastraea irregularis* and *Pocillopora verrucosa* exposed to experimental thermal stress

3.1 Abstract

Mass coral bleaching events caused by ocean warming have become more frequent and are predicted to increase in frequency and severity in the coming years. The degree of coral bleaching, recoverability, and mortality is highly variable and considered to be affected by the coral's antioxidant capacity. Understanding the antioxidant enzyme activity of different species of corals from varying environmental regimes when exposed to prolonged thermal stress and following the cessation of thermal stress yields information that can be used to inform better conservation measures. This study investigated the antioxidant enzyme activity of *Anomastraea irregularis* (massive morphology) and *Pocillopora verrucosa* (branching morphology) hosts from environmentally variable intertidal and more stable subtidal habitats from the east coast of South Africa. The corals were maintained in closed recirculating aquaria and exposed to two thermal stress conditions (control: 26°C, thermal stress treatments: 28°C and 30°C) for three months, and then they were maintained at control conditions for two months to monitor recovery. Commercial kits were used to measure the protein concentrations, superoxide dismutase, catalase, glutathione peroxidase, glutathione s-transferase, and caspase 3 activity in coral tissue that was collected at three points: At the start of thermal stress, the end of thermal stress, and the end of recovery. Both thermal stress treatments caused higher protein concentrations and antioxidant enzyme activities (superoxide dismutase, catalase, and glutathione peroxidase) in both species from both habitats. Additionally, there were inherent differences between the species and habitat, highlighting that thermal regimes of habitats and coral species can influence biochemical activity. The protein concentrations were much higher and antioxidant enzyme activities (superoxide dismutase, catalase, and glutathione peroxidase) much lower in intertidal fragments than subtidal fragments and *A. irregularis* than *P. verrucosa* when thermally stressed. The higher protein concentrations in these corals may have supported their physiological processes, thereby requiring less antioxidant activities due to less oxidative stress. Neither superoxide dismutase, catalase, glutathione peroxidase nor caspase 3 activities of either species or habitat decreased to control levels at the end of recovery, suggesting that the biochemical processes in these corals require a longer period for a full recovery.

Keywords: heat stress, protein, bleaching, apoptosis, oxidative stress

3.2 Introduction

Scleractinian reef-building corals and their symbiosis with unicellular algae of the family Symbiodiniaceae create coral reefs, which are some of the largest and most diverse ecosystems on the planet (Brown 1997; Stat et al. 2012). They are found in more than 100 countries and territories and despite only covering approximately 0.2% of the seafloor they have been estimated to support more than 25% of marine biodiversity (Bahn et al. 2002) and over 500 million people through socio-economic and ecosystem services (Wilkinson 2004; Bellantuono et al. 2012a; Hughes et al. 2017a; Eddy et al. 2021). The great productivity of coral reefs in nutrient-deficient waters is due to the Symbiodiniaceae yielding the coral hosts photosynthetically produced carbohydrates and oxygen in exchange for shelter and inorganic nutrients (Muscatine and Porter 1977; Dubinsky and Jokiel 1994; Lough 2008; Osinga et al. 2012). This symbiosis is, however, delicate, and when there are environmental perturbations or biological stressors, the coral expels the Symbiodiniaceae, or there is a loss in the photosynthetic pigment of the Symbiodiniaceae in a phenomenon termed coral bleaching (Baker et al. 2008; Wooldridge 2017).

Before the 1980s, coral bleaching was only documented in a few locations and considered to be due to localised water quality issues (Hoegh-Guldberg et al. 2018). Since then, several global mass coral bleaching events have occurred and have been correlated with the steadily increasing ocean temperatures and thermal anomalies (Hughes et al. 2018a; Tebbett et al. 2023). Before the 1990s, global coral cover records were scant, but from 1997 to 2018, global coral cover has been estimated to have dropped by 17%, with the most losses recorded in the Western Atlantic and Central Pacific regions (Tebbett et al. 2023). Mass coral bleaching events are predicted to increase in frequency and severity with ocean warming and increased thermal anomalies (Hoegh-Guldberg et al. 2023). Reef-building corals are pivotal to the functioning and productivity of coral reef ecosystems hence a significant loss of corals has broadscale negative effects on the biota that inhabit the system and on coral reef users (Hoegh-Guldberg et al. 2018; McLeod et al. 2021).

However, not all corals respond equally to thermal stress and field observations and laboratory experiments have shown that coral bleaching severity and mortality are highly heterogenous (Kuffner et al. 2015; Chapron et al. 2022; Brown et al. 2023; Hoegh-Guldberg et al. 2023; Kuo et al. 2023). The cellular mechanisms responsible for the breakdown in the symbiosis resulting in coral bleaching still require investigation to understand the variable responses (Weis 2008; Mydlarz et al. 2010; Nielsen et al. 2018). Coral bleaching is commonly explained by the

oxidative theory of bleaching (Lesser 1997; Downs et al. 2002; Weis 2008). According to this theory, increased temperature disrupts the photosynthetic ability of the Symbiodiniaceae to efficiently convert light energy into chemical energy, which leads to the production of reactive oxygen species such as hydrogen peroxide (H_2O_2), oxygen singlets ($^1O_2$), hydroxyl radical ($OH^{\bullet}$), or superoxide anions ($O_2^{\bullet-}$) which leaks into the coral host cells where it causes damage to the hosts lipids, proteins, and deoxyribose nucleic acid (DNA) (Lesser 1997; Downs et al. 2002; Weis 2008; Wall et al. 2021). As a final defence mechanism, the coral hosts are believed to eject the Symbiodiniaceae cells to prevent mortal cellular and tissue damage (Downs et al. 2002). However, below a certain reactive oxygen species concentration threshold, there are several antioxidant enzymes and proteins in the coral host cells that can prevent and ameliorate oxidative damage, thereby influencing the corals' resilience/susceptibility to bleaching and mortality when faced with thermal stress (Downs et al. 2000; Yakovleva et al. 2004; Baird et al. 2009; Fitt et al. 2009; Wall et al. 2018; Dias et al. 2019b; Tisthammer et al. 2021).

In the past decade, the symbiosis between the coral host and Symbiodiniaceae has been re-examined, and it is suggested to not be entirely mutualistic (Wooldridge 2010; Matz 2024). Evidence suggests that under normal circumstances, the coral hosts may be controlling the Symbiodiniaceae reproduction by limiting the amount of nitrogen passed to the algae while passing on carbon dioxide freely thereby receiving continual supply of photosynthates (Matz 2024). Therefore, alternative hypotheses suggest that corals bleaching does not result solely from oxidative damage but may result from metabolic and nutrient cycling imbalances within the symbiosis, with the hosts stress being a defining factor (Wooldridge 2009; Matz 2024; Schlotheuber et al. 2024). During thermal stress, elevated respiration rates and reduced photosynthetic carbon fixation may lead to the host not receiving enough energy to sustain the Symbiodiniaceae populations thereby promoting bleaching (Wooldridge 2009). Additionally, during thermal stress the impairment of nutrients like nitrogen and phosphorus cycling by the host may cause an increase in Symbiodiniaceae growth rates due to increased availability of nutrients (Matz 2024). This can cause a shift in the symbiosis with the Symbiodiniaceae becoming parasitic as the algae move towards prioritising their own growth over translocating photosynthates to the coral host (Wooldridge 2009; Matz 2024). Bleaching may result as a way for the host to lower the metabolic burden before severe oxidative stress can occur (Matz 2024). Altogether the literature shows that coral bleaching may not always be a simple breakdown of mutualism due to oxidative stress, but rather a complex interplay of oxidative damage, carbon limitation and imbalances in nutrient cycling. This highlights the need for studies to integrate

biochemical, metabolic and physiological markers when assessing coral thermal stress responses.

Superoxide dismutase, catalase, and glutathione peroxidase are three major scavenger enzymes in the host that usually increase in activity with increased thermal stress to inhibit reactive oxygen species to reduce cellular damage (Downs et al. 2002; Yakovleva et al. 2004; Baird et al. 2009; Hawkins et al. 2015). Superoxide dismutase is considered the first line of defence against oxidative stress and converts superoxide anions to hydrogen peroxide and water (Anithajothi et al. 2014). Catalase follows, converting the hydrogen peroxide to water and oxygen, while glutathione peroxidase also reduces hydrogen peroxide and other organic peroxides (Anithajothi et al. 2014). Glutathione s-transferase is an enzyme involved in the detoxification process by acting on electrophilic xenobiotics and thereby protects the cells against mutagenic, carcinogenic, and toxic effects of compounds and also performs cell macrostructural repair by removing DNA hydroperoxides and lipid peroxides (Limon-Pacheco and Gonsebatt 2009). Caspase 3 are proteases that are involved in the process of apoptosis, where cells are programmed to die if they are irreversibly damaged (Chowdhury et al. 2008), and the caspase 3 activity in the coral host usually increases when stressed and can be detected much earlier than signs of bleaching (Ros et al. 2016). Corals also possess other proteins, such as heat shock proteins 70, 60, and 32, that are upregulated to ameliorate the effects of thermal stress (Seveso et al. 2019). In addition, proteins are also an important energy source after lipids and carbohydrates and therefore their concentrations can indicate the corals health status (Rodrigues and Grottoli 2007).

Since these biochemical biomarkers are integral in mitigating oxidative stress and indicate the coral health status, they have previously been measured in coral thermal stress studies and have been found to vary in corals with different thermal histories: Corals from thermally variable habitats have been found to rapidly increase antioxidant activity when exposed to thermal stress, thereby mitigating oxidative damage (Majerová and Drury 2022). Moreover, coral species from thermally variable habitats can also exhibit different patterns of antioxidant activity (Majerová and Drury 2022). Furthermore, if alternative bleaching hypotheses such as carbon limitation or nutrient imbalances play a role, antioxidant enzyme responses may not fully correlate with observed bleaching patterns. This highlights the need to study the activity of these enzymes in different coral species and from locations that experience different thermal regimes to acquire rudimentary knowledge of how these antioxidant enzymes function in animals in thermally stable and fluctuating habitats in conjunction with physiological assays.

Additionally, understanding how antioxidant enzyme activity responds to prolonged thermal stress is also important because thermal damage is progressive and cumulative over time (Dias et al. 2019b). There is, furthermore, a need to understand how the antioxidant activity changes during the recovery period after thermal stress ceases. Therefore, these biochemical biomarkers were investigated in a massive species, *Anomastraea irregularis*, and a branching species, *Pocillopora verrucosa*, from the environmentally variable intertidal pools and environmentally stable subtidal habitat from Treasure Beach, South Africa, exposed to six months of experimental thermal stress and two months of recovery. Since the intertidal corals and *A. irregularis* species were considered thermally resilient, it was hypothesised that they would have higher baseline antioxidant activities and at the start of heat stress the levels would rise and then decrease at the end of the study.

3.3 Materials and methods

3.3.1 Sample preparation

The coral collection, experimental design, tissue extraction, and surface area measurements were detailed in Chapter 2. Care was taken to use sterilised equipment and working surfaces during the processing and analysing of the samples to minimise contamination. The tubes containing the two millilitres of coral host tissue were removed from the -80°C-freezer and thawed on ice. Then 375 µL of each sample was transferred to a new two-millilitre centrifuge tube containing 1125 µL of an extraction buffer (0.42 mol L^{-1} NaCl, 1 mol L^{-1} $MgCl_2$, 1 mol L^{-1} HEPES, 0.5 mol L^{-1} EDTA, and 25 % (v/v) glycerol) (modified from Mosser et al. 1988) and a stainless-steel bead. The samples were then lysed in a tissue lyser (TissueLyser LT, Qiagen, China) at 50 oscillations for two minutes. The stainless-steel beads were removed from the tubes. The lysed samples were then centrifuged at 10000 x g for 15 minutes. The supernatant from each tube was then removed and transferred to a new 1.5-mL-micro-tube and stored in the -80°C-freezer until further protein and enzymatic analyses were performed.

3.3.2 Protein concentration

The commercial Pierce BCA total protein assay kit (ThermoFisher Scientific, Germany, Catalogue number: 23225) was used and performed to the manufacturer's specifications to determine the protein concentrations of the samples. Duplicates of each sample (50:50 dilution factor) and a standard series of bovine serum albumin of concentrations ranging from 0-2 mg mL^{-1} were added to 96-well microtiter plates. The working reagent, comprising 50 parts reagent A and one part Reagent B, was then added to each well, and the plates were incubated at 37°C for 30 minutes. The plates were then cooled to room temperature and shaken. Thereafter, the

absorbance for each well was measured at 562 nm using a multi-well plate reader (PowerWave XS, Biotek, United States of America). Protein concentrations of the samples were attained using the standard curve generated from the absorbances of the standard albumin series. The dilution factor, volume of sample, and surface area of the fragments were used to normalise the protein concentration (expressed as mg cm^{-2}). It is noteworthy that these are not total protein concentrations, but total host protein concentrations.

3.3.3 Enzymatic assays

Before enzyme activity assays were conducted, the dynamic range for the sample set was determined for each assay, using at least two samples of each species from each habitat and from each treatment. Sample dilutions were then made accordingly, as reported below.

3.3.3.1 Superoxide dismutase activity

A commercial kit (Sigma-Aldrich, Germany, Catalogue number: 19160), which utilises nitroblue tetrazolium and xanthine oxidase, was used to determine the superoxide dismutase activity in the samples. Duplicates of each sample and a standard series of superoxide dismutase solution (concentrations ranging from 0.001-200 U mL^{-1}) were added to 96-well microtiter plates. The working solution, enzyme working solution, and dilution buffer were then added to appropriate wells. Positive, negative, and colour control wells were also set up according to the manufacturer's protocol. The plates were incubated at 37°C for 20 minutes and the absorbance for each well was measured at 450 nm using a multi-well plate reader. The superoxide dismutase activity (inhibition rate %) in the samples was calculated using the equation:

$$\left(\frac{(Abs_{450} of\ positive\ control - Abs_{450} of\ negative\ control) - (Abs_{450} of\ sample - Abs_{450} of\ colour\ control)}{((Abs_{450} of\ positive\ control - Abs_{450} of\ negative\ control))}\right) \times 100.$$

The enzyme activity was normalised to the protein concentration of the sample and expressed as % inhibition mg^{-1} of protein.

3.3.3.2 Catalase activity

A commercial colorimetric assay kit (Sigma-Aldrich, Germany, Catalogue number: CAT100), which utilises peroxidic function of catalase, was used for determining the catalase activity in the samples. The manufacturer's colorimetric assay protocol was followed using duplicates of each sample (50x dilution factor), a standard series of hydrogen peroxide solution of concentrations ranging from 0-7.5 mmol L^{-1}, and the appropriate reagents were added to

microcentrifuge tubes for the colorimetric reaction to occur. Following the colour development, 350 µL of the solutions were pipetted to 96-well microtiter plates and the absorbances were measured at 560 nm using a multi-well plate reader. The amount of hydrogen peroxide remaining in the colorimetric reaction mixture was obtained using the hydrogen peroxide standard curve. The difference in the µmoles of hydrogen in the negative control and in the samples (Δµmoles of hydrogen peroxide) were calculated and the catalase activity (µmoles min^{-1} ml^{-1}) was then calculated using the equation:

$$\frac{\Delta \mu moles\ of\ hydrogen\ peroxide\ \times d\ \times 100}{V\ \times t}$$

where d is the dilution factor of the sample, V is the volume (mL) of the sample used in the reaction, and t is the duration of the reaction (min). The catalase activity was normalised to the protein concentration of the sample (µmoles min^{-1} ml^{-1} mg^{-1} of protein).

3.3.3.3 Glutathione peroxidase activity

A commercial kit (Sigma-Aldrich, Germany, Catalogue number: CGP1) was used to determine the glutathione peroxidase activity in the samples. The kit indirectly determines the activity of glutathione peroxidase by measuring the decrease in NADPH absorbance at 340 nm during the oxidation of NADPH to $NADP^+$, since glutathione peroxidase is a rate limiting factor in the coupled reactions. The manufacturer's protocol was followed using duplicates of each sample (50x dilution factor); however, the appropriate reagents and the samples volumes were scaled down (so that the total volume in each well was 350 µL) to be added to 96-well microtiter plates instead of cuvettes. The absorbances were measured at 340 nm using a multi-well plate reader every 10 seconds for a minute. The glutathione peroxidase activity (mmol min^{-1} mL^{-1}) was calculated using the equation:

$$\frac{(Abs_{340}\ per\ minute\ of\ blank - Abs_{340}\ per\ minute\ of\ sample)\ \times d}{\varepsilon mM \times V}$$

where d is the dilution factor of the sample, εmM is 6.22 the molar extinction coefficient for NADPH and V is the volume (mL) of the sample used in the reaction. The glutathione peroxidase activity was normalised to the protein concentration of the sample (mmol min^{-1} ml^{-1} mg^{-1} of protein).

3.3.3.4 Glutathione s-transferase activity

A commercial kit (Sigma-Aldrich, Germany, Catalogue number: CS0410) was used to determine the glutathione s-transferase activity in the samples. The kit determines the activity

of glutathione s-transferase by measuring the increase in absorbance of the enzyme substrate 1-chloro-2,4-dinitrobenze at 340 nm when it conjugates with the thiol group of glutathione. The manufacturer's protocol for the assay in 96-well microtiter plates was followed using undiluted duplicates of each sample. The absorbances were read at 340 nm using a multi-well plate reader every minute for six minutes. The glutathione s-transferase activity (mmol min^{-1} mL^{-1}) was calculated using the equation:

$$\frac{\left(\left(\frac{final - initial\ Abs_{340}\ of\ sample}{reaction\ time}\right) - \left(\frac{final - initial\ Abs_{340}\ of\ blank}{reaction\ time}\right)\right) \times V \times d}{\text{ƐmM} \times \text{Venzyme}}$$

where V is the reaction volume in the 96-well plate, d is the dilution factor of the sample, ƐmM is the molar extinction coefficient for 1-chloro-2,4-dinitrobenze of 5.3 εmM (mM^{-1} cm^{-1}) after correction for the microplate wells path length, and Venzyme is the volume (mL) of the sample used in the reaction. The glutathione s-transferase activity was normalised to the protein concentration of the sample and expressed in mmol min^{-1} ml^{-1} mg^{-1} of protein.

3.3.3.5 Caspase 3 activity

A commercial colorimetric assay kit (Sigma-Aldrich, Germany, Catalogue number: CASP-3-C) was used to determine the caspase 3 activity in the samples. The kit determines the activity of caspase 3 by measuring the increase in absorbance of the peptide substrate p-nitroaniline (pNA) at 405 nm. The manufacturer's protocol for the assay in 96-well microtiter plates was followed using undiluted duplicates of each sample and a standard series of pNA solution of concentrations ranging from 10-200 µmol L^{-1}. The plates were incubated at 37°C for 90 minutes and the absorbance were measured at 405 nm. The amount of pNA (µmol) in the samples was obtained using the standard series calibration curve generated from the absorbances of the standard pNA versus the concentrations of pNA. The caspase 3 activity (µmol pNA min^{-1} ml^{-1}) was calculated using the equation:

$$\frac{Amount\ of\ pNA \times d}{t \times V}$$

where d is the dilution factor of the sample, t is the reaction time (minutes), and V is the volume of the sample (mL). The caspase 3 inhibited and uninhibited activity (**Appendix 8**) was subtracted and normalised to the protein concentration of the sample and multiplied by 1000 to express it in nmol pNA min^{-1} ml^{-1} mg^{-1} of protein.

3.3.4 Statistical analysis

To assess if there were significant differences in protein concentration, superoxide dismutase activity, catalase activity, glutathione peroxidase activity, glutathione s-transferase activity, and caspase 3 activity between habitats (intertidal and subtidal) and temperature treatments (control (26°C), 28°C, and 30°C) at the different time points (before thermal stress, end of thermal stress, and end of recovery) six Generalised Linear Models (GLM) were executed for each species. The residuals of the dependent variables were nonparametric (one sample Kolmogorov-Smirnov on residuals $p < 0.05$) therefore GLMs were performed with a gamma distribution with log link functions. The colony effect and tank replicate were added as covariates to account for random effects but were removed from the models if they were insignificant. Additionally, Bonferroni pairwise comparisons using estimated marginal means were performed to determine statistical differences within tested groups.

3.4 Results

3.4.1 Protein concentration

The protein concentration of all control fragments did not significantly differ over the duration of the study (**Figure 3.1**). The protein concentrations of *A. irregularis* fragments differed significantly between the treatments (**Figure 3.1** and **Table 3.1**). Overall, the protein concentrations of intertidal *A. irregularis* fragments in the 28°C and 30°C treatments were ~1% higher than their conspecifics in the controls (**Figure 3.1** and **Appendix 9**). Overall, the protein concentrations of the subtidal *A. irregularis* fragments in the 28°C and 30°C treatments were ~4% and ~5% significantly higher than their conspecifics in the controls (**Figure 3.1** and **Appendix 9**). There was a significant interaction effect of time x habitat and habitat x treatment for the protein concentration of *P. verrucosa* fragments (**Table 3.1**). The Bonferroni pairwise analysis indicated exactly where the significant differences occurred and the mean differences between tested pairs (**Appendix 9**). In general, protein concentration in subtidal *P. verrucosa* in the 28°C treatment and the 30°C treatment declined significantly by ~5% and ~ 6% from the beginning to the end of the study (**Figure 3.1**, **Table 3.1**, and **Appendix 9**). However, protein concentrations were elevated in intertidal *P. verrucosa* by ~2% in the 28°C and ~4% in the 30°C treatments over the same period (**Figure 3.1**, **Table 3.1**, and **Appendix 9**). Furthermore, the control subtidal *P. verrucosa* had the highest protein concentrations (mean 5.97 ± 0.17 standard deviation), followed by the 28°C treatment (mean 5.90 ± 0.20 standard deviation) and 30°C treatment (mean 5.79 ± 0.21 standard deviation) (**Figure 3.1**, **Table 3.1**, and **Appendix 9**). In contrast, protein concentrations in intertidal *P. verrucosa* were highest in the 30°C

treatment (mean 6.43 ± 0.21 standard deviation) followed by the 28°C treatment (mean 6.26 ± 0.21 standard deviation) and lowest in the control fragments (mean 6.16 ± 0.16 standard deviation) (**Figure 3.1**, **Table 3.1**, and **Appendix 9**). Overall, intertidal *A. irregularis* had ~23% and intertidal *P. verrucosa* had ~7% significantly higher protein concentrations than their subtidal conspecifics (**Figure 3.1**, **Table 3.1**, and **Appendix 9**). Overall, the protein concentrations of *A. irregularis* were also ~108% higher than *P. verrucosa* fragments (**Figure 3.1**).

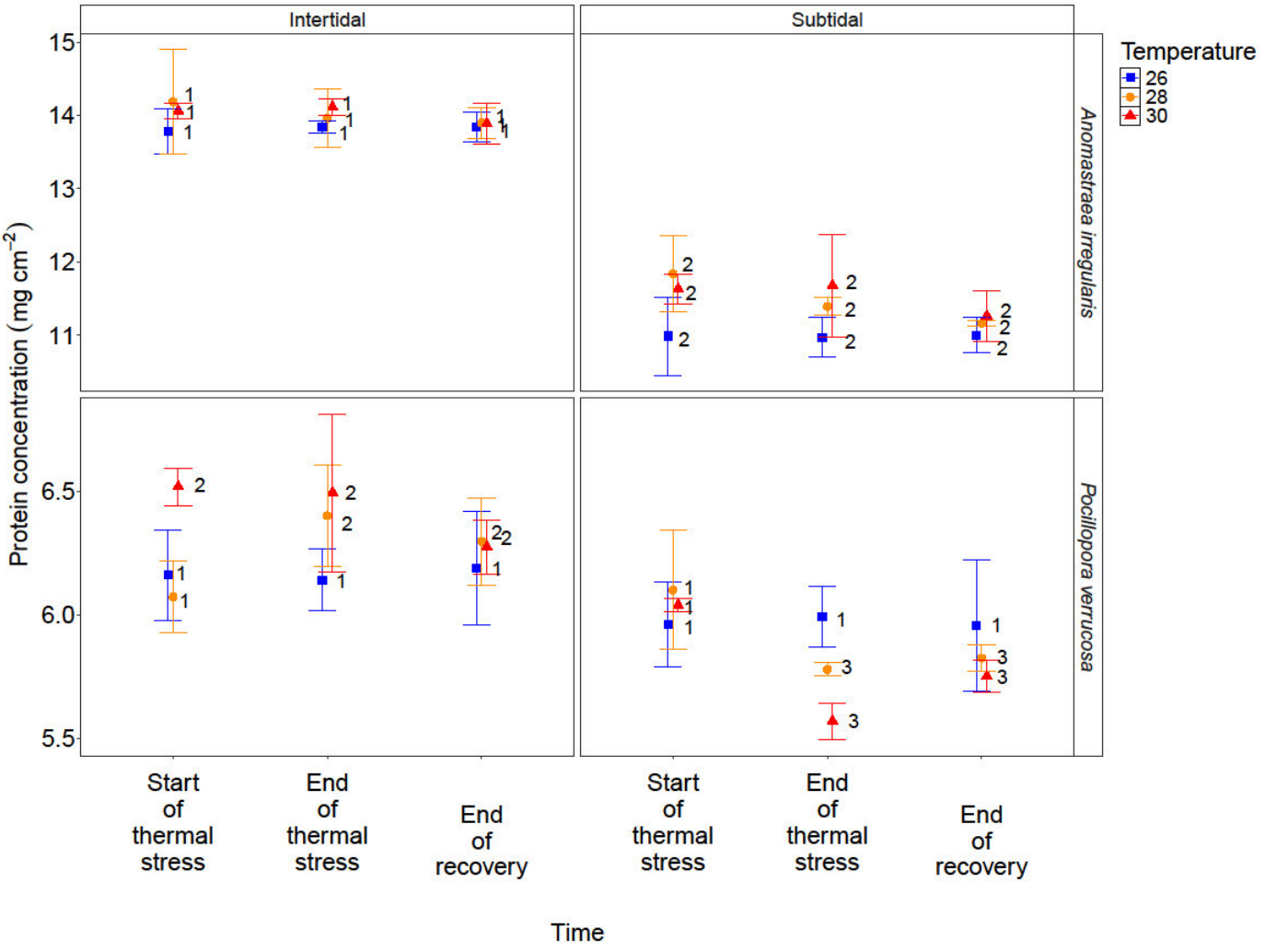

Figure 3.1: Average total host protein concentration (mg cm^{-2}) ± 1 standard deviation in *Anomastraea irregularis* and *Pocillopora verrucosa* fragments from the intertidal and subtidal habitats at the start (31 Jul) and end of thermal stress (23 Oct) and end of recovery (20 Dec). Numbers (1-3) indicate significant differences between tested groups (time*temperature*habitat) for each species according to Bonferroni pairwise tests for the GLM.

3.4.2 Superoxide Dismutase activity

The superoxide dismutase activity of all control fragments did not significantly differ throughout the study (**Figure 3.2**). The superoxide dismutase activity of both species from both habitats in both thermal stress treatments significantly differed throughout the study (**Figure 3.2**, **Table 3.1**, and **Appendix 10**). At the start of the thermal stress, the superoxide dismutase activity of both species from both habitats in both treatments was significantly higher than their conspecifics in the control (**Figure 3.2**, **Table 3.1**, and **Appendix 10**). At the start, the elevation in superoxide dismutase activity between the control-28°C and the control-30°C was ~1512% and ~1611% for subtidal *A. irregularis*, ~1396% and ~1715% for intertidal *A. irregularis*, ~2482% and ~2692% for subtidal *P. verrucosa* and ~2560% and ~2422% for intertidal *P. verrucosa* (**Figure 3.2**). The activity of the thermally stressed fragments decreased at the end of the thermal stress and recovery period but remained significantly higher than the control fragments (**Figure 3.2**, **Table 3.1**, and **Appendix 10**). At the end of recovery, the elevation in superoxide dismutase activity between the control-28°C and the control-30°C was ~1135% and ~1145% for subtidal *A. irregularis*, ~881% and ~993% for intertidal *A. irregularis*, ~1834% and ~1888% for subtidal *P. verrucosa* and ~1594% and ~1725% for intertidal *P. verrucosa* (**Figure 3.2**). In general, the superoxide dismutase activity of *A. irregularis* from both habitats in the 30°C treatment was ~10% significantly higher than their conspecifics in the 28°C treatment (**Figure 3.2**, **Table 3.1**, and **Appendix 10)**. The superoxide dismutase activity of subtidal *P. verrucosa* in the 30°C treatment was ~6% significantly higher than their conspecifics in the 28°C treatment, but the activity of the intertidal *P. verrucosa* in the 28°C treatment was ~3% significantly higher than their conspecifics in the 30°C (**Figure 3.2**, **Table 3.1**, and **Appendix 10)**. Overall, the intertidal fragments of *A. irregularis* and *P. verrucosa* had ~26% and ~7% significantly lower superoxide dismutase activity than their subtidal conspecifics (**Figure 3.2**, **Table 3.1**, and **Appendix 10**). Overall, the superoxide dismutase activity of *A. irregularis* in the two thermal stress treatments was ~43% lower than the *P. verrucosa* fragments (**Figure 3.2**).

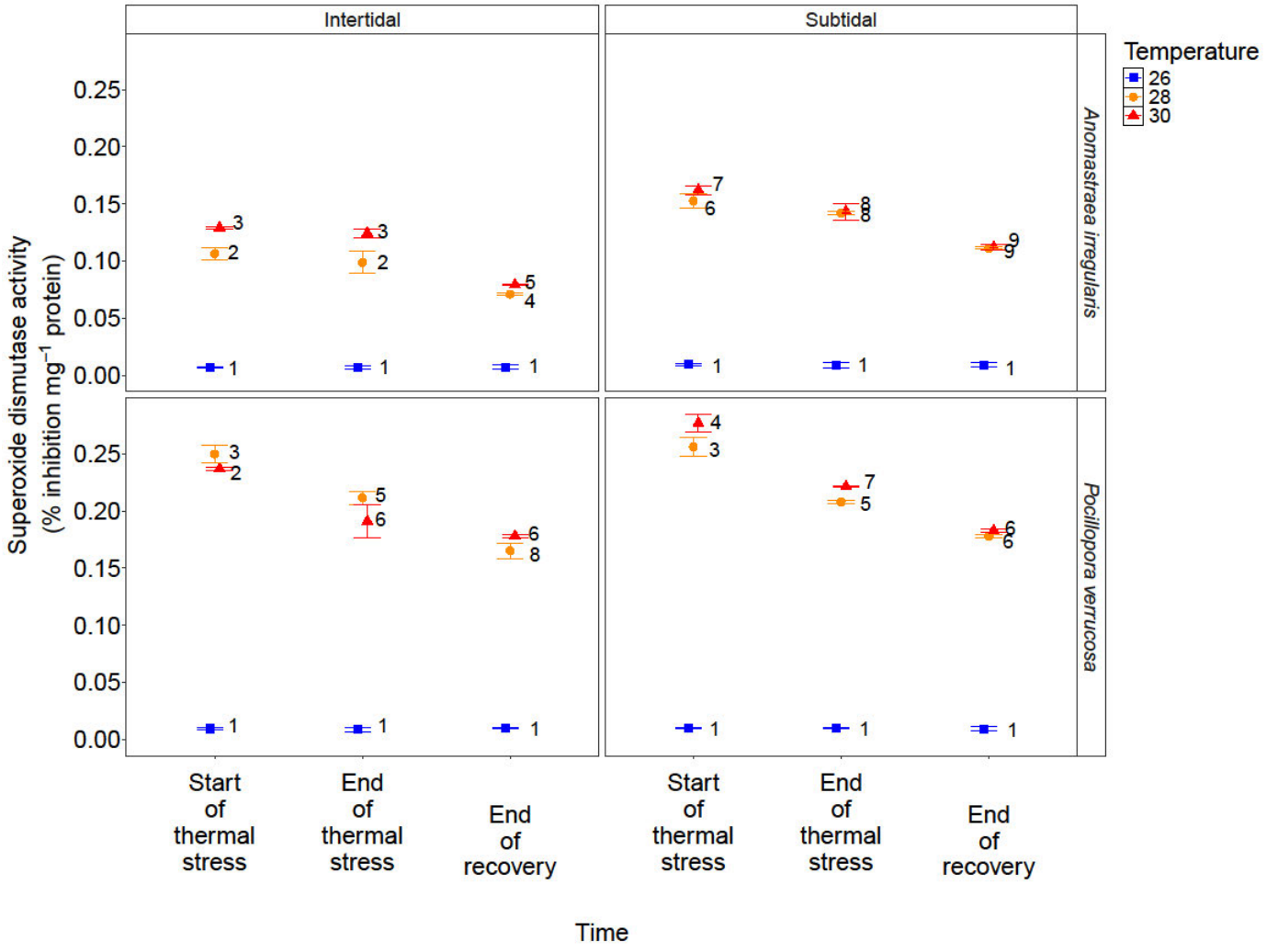

Figure 3.2: Average Superoxide dismutase activity (% inhibition mg^{-1} of total host protein concentration) ± 1 standard deviation in *Anomastraea irregularis* and *Pocillopora verrucosa* fragments from the intertidal and subtidal habitats at the start (31 Jul) and end of thermal stress (23 Oct) and end of recovery (20 Dec). Numbers (1-9) indicate significant differences between tested groups (time*temperature*habitat) for each species according to Bonferroni pairwise tests for the GLM.

3.4.3 Catalase activity

The catalase activity of all control fragments did not significantly differ throughout the study (**Figure 3.3**). The catalase activity of both species from both habitats in both thermal stress treatments significantly differed throughout the study (**Figure 3.3**, **Table 3.1**, and **Appendix 11**). At the start of thermal stress, the catalase activity of both species from both habitats in both treatments was significantly higher than their conspecifics in the control, the activity of the thermally stressed fragments then decreased at the end of the thermal stress and recovery period but remained significantly higher than the control fragments (**Figure 3.3**, **Table 3.1**, and

Appendix 11). At the start, the increase in catalase activity between the control-28°C and the control-30°C was ~136% and ~201% for subtidal *A. irregularis*, ~369% and ~503% for intertidal *A. irregularis*, ~233% and ~289% for subtidal *P. verrucosa* and ~495% and ~514% for intertidal *P. verrucosa* (**Figure 3.3**). At the end of recovery, the increase in catalase activity between the control-28°C and the control-30°C was ~71% and ~80% for subtidal *A. irregularis*, ~92% and ~186% for intertidal *A. irregularis*, ~65% and ~98% for subtidal *P. verrucosa* and ~248% and ~316% for intertidal *P. verrucosa* (**Figure 3.3**). Overall, the catalase activity of *A. irregularis* and *P. verrucosa* in the 30°C treatment was ~28% and ~14% significantly higher than their conspecifics in the 28°C treatment (**Figure 3.3**, **Table 3.1**, and **Appendix 11)**. Overall, the catalase activity of intertidal fragments of *A. irregularis* was ~19% and *P. verrucosa* was ~10% significantly lower than their subtidal conspecifics (**Figure 3.3**, **Table 3.1**, and **Appendix 11**). Overall, the catalase activity of *A. irregularis* was ~27% lower than *P. verrucosa* (**Figure 3.3**).

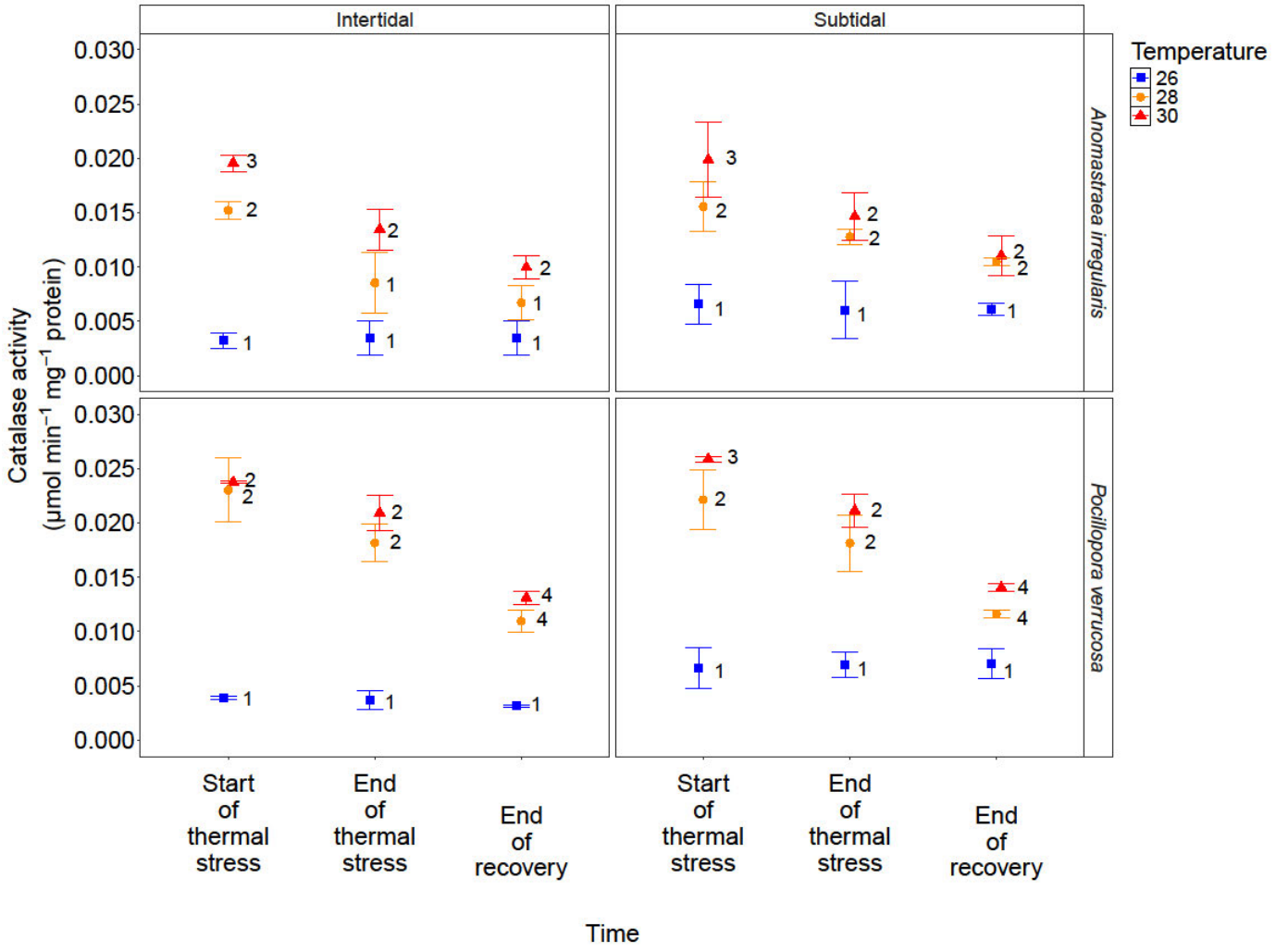

Figure 3.3: Average Catalase activity (µmol min^{-1} mg^{-1} of total host protein) ± 1 standard deviation in *Anomastraea irregularis* and *Pocillopora verrucosa* fragments from the intertidal and subtidal habitats at the start (31 Jul) and end of thermal stress (23 Oct) and end of recovery (20 Dec). Numbers (1-4) indicate significant differences between tested groups (time*temperature*habitat) for each species according to Bonferroni pairwise tests for the GLM.

3.4.4 Glutathione peroxidase activity

The glutathione peroxidase activity of all control fragments did not significantly differ throughout the study (**Figure 3.4**). The glutathione peroxidase activity of both species from both habitats in both thermal stress treatments significantly differed throughout the study (**Figure 3.4**, **Table 3.1**, and **Appendix 12**). At the start of thermal stress, the glutathione peroxidase activity of both species from both habitats in both treatments was significantly higher than their conspecifics in the control, the activity of the thermally stressed fragments then decreased at the end of the thermal stress and recovery period but remained significantly

higher than the control fragments (**Figure 3.4**, **Table 3.1**, and **Appendix 12**). At the start, the elevation in glutathione peroxidase activity in the 28°C and the 30°C treatments compared to the control was ~1604% and ~8491% for subtidal *A. irregularis*, ~6448% and ~8879% for intertidal *A. irregularis*, ~5254% and ~11748% for subtidal *P. verrucosa* and ~7748% and ~10094% for intertidal *P. verrucosa* (**Figure 3.4**). At the end of recovery, the elevated glutathione peroxidase activity in the 28°C and 30°C treatments compared to the control was ~957% and ~3612% for subtidal *A. irregularis*, ~4671% and ~5628% for intertidal *A. irregularis*, ~3871% and ~4635% for subtidal *P. verrucosa* and ~5262% and ~6054% for intertidal *P. verrucosa* (**Figure 3.4**). In general, the glutathione peroxidase activity of *A. irregularis* and *P. verrucosa* in the 30°C treatment was ~117% and ~48% significantly higher than their conspecifics in the 28°C treatment (**Figure 3.4**, **Table 3.1**, and **Appendix 12)**. Overall, the intertidal fragments of *A. irregularis* and *P. verrucosa* had ~6% and ~30% significantly lower glutathione peroxidase activity than their subtidal conspecifics (**Figure 3.4**, **Table 3.1**, and **Appendix 12**). Overall, the glutathione peroxidase activity of *A. irregularis* was ~34% lower than *P. verrucosa* (**Figure 3.4**).

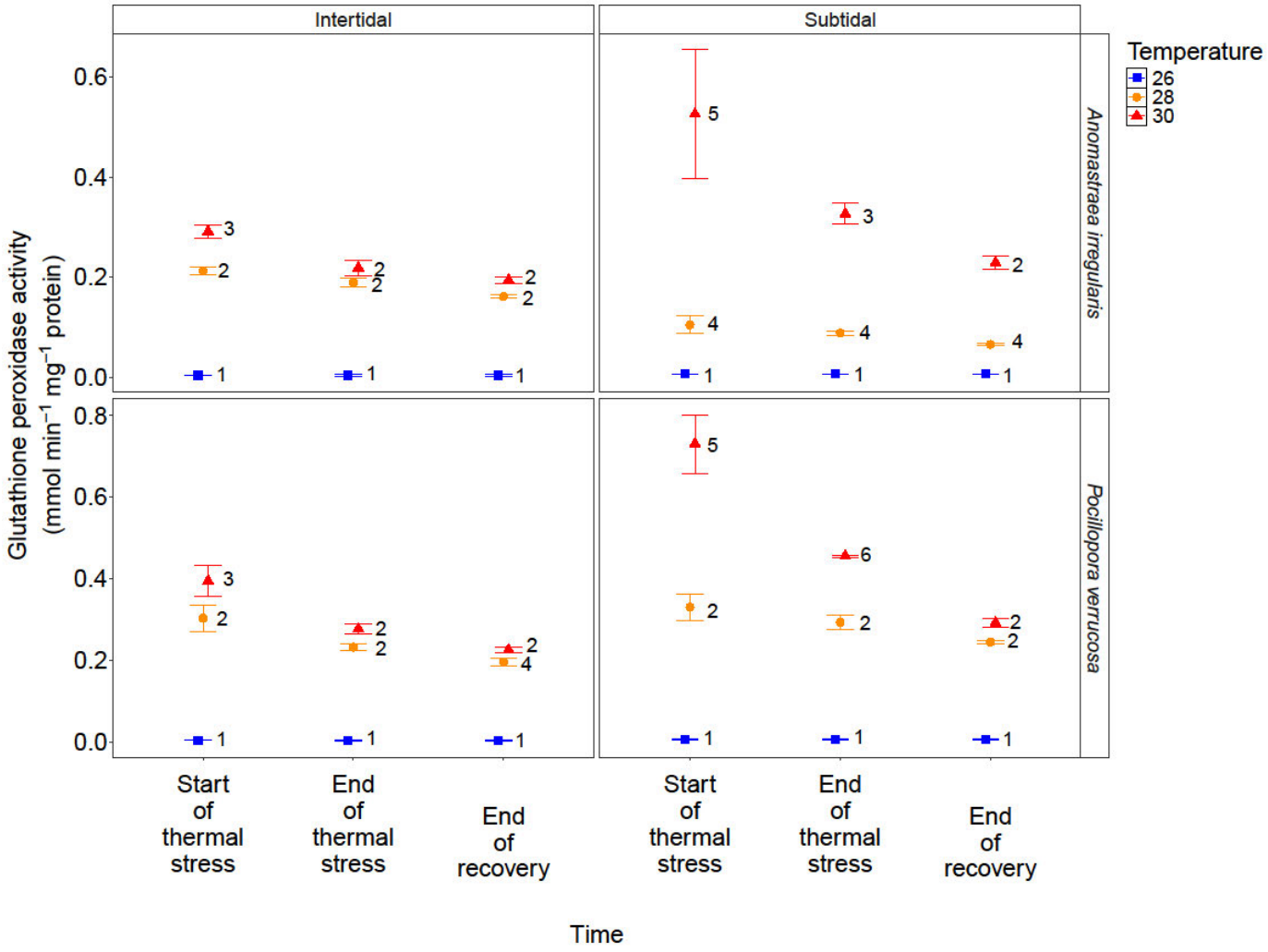

Figure 3.4: Average Glutathione peroxidase activity (mmol min^{-1} mg^{-1} of total host protein) ± 1 standard deviation in *Anomastraea irregularis* and *Pocillopora verrucosa* fragments from the intertidal and subtidal habitats at the start (31 Jul) and end of thermal stress (23 Oct) and end of recovery (20 Dec). Numbers (1-6) indicate significant differences between tested groups (time*temperature*habitat) for each species according to Bonferroni pairwise tests for the GLM.

3.4.5 Glutathione s-transferase activity

The glutathione s-transferase activity of all control fragments did not significantly differ throughout the study (**Figure 3.5**). Overall, the glutathione s-transferase activity of *A. irregularis* in the 30°C treatment was ~7% significantly higher than their conspecifics in the 28°C treatment and ~13% than those in the control (**Figure 3.5**, **Table 3.1**, and **Appendix 13)**. Similarly, the glutathione s-transferase activity of *P. verrucosa* in the 30°C treatment was ~26% significantly higher than conspecifics in the 28°C treatment and ~29% than those in the control (**Figure 3.5**, **Table 3.1**, and **Appendix 13)**. Overall, the glutathione s-transferase

activity of both species did not significantly differ between habitats (**Figure 3.5**, **Table 3.1**). Overall, the glutathione s-transferase activity also did not differ between the two species (**Figure 3.5**).

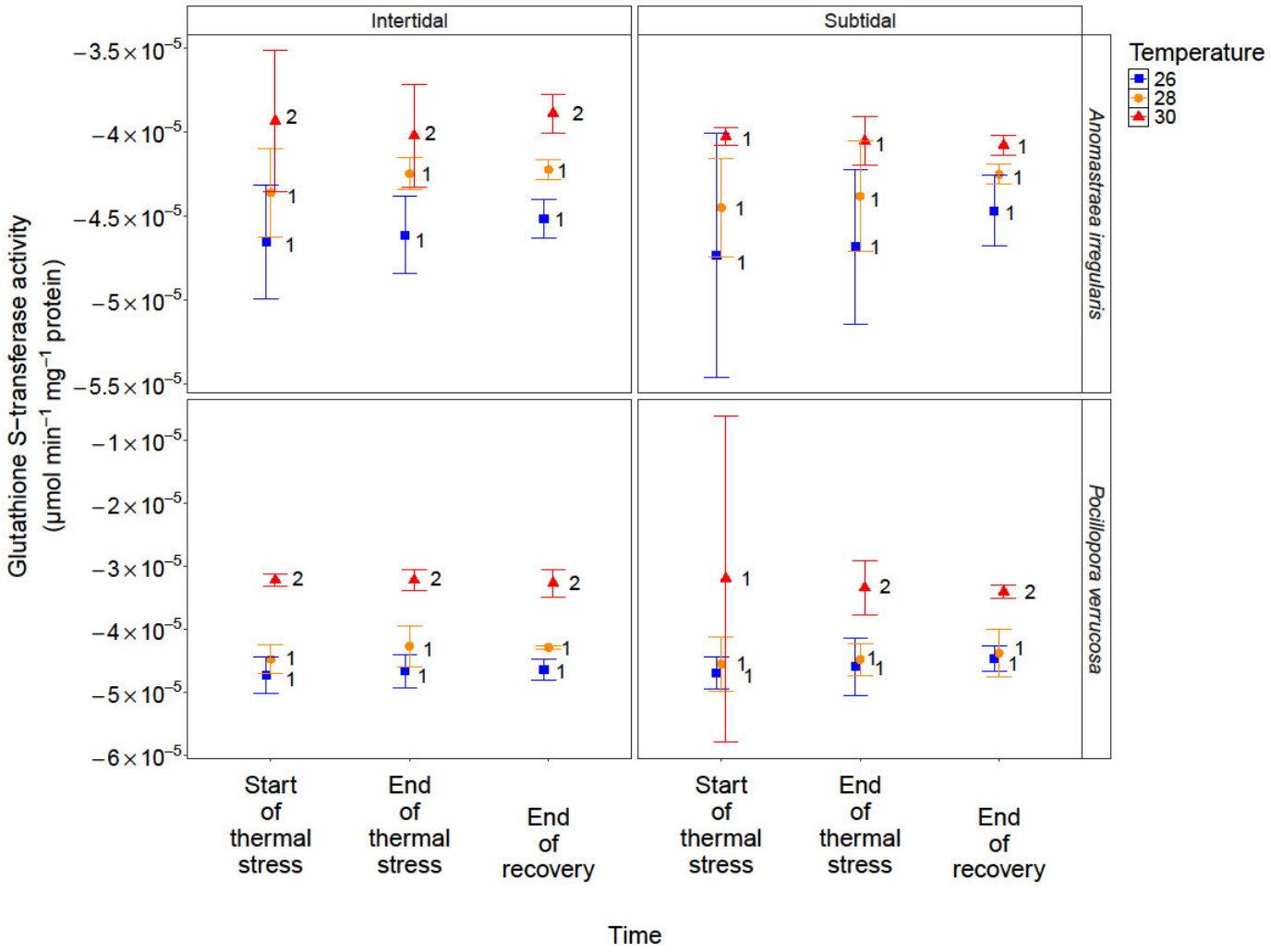

Figure 3.5: Average Glutathione S-transferase activity (µmol min^{-1} mg^{-1} of total host protein) ± 1 standard deviation in *Anomastraea irregularis* and *Pocillopora verrucosa* fragments from the intertidal and subtidal habitats at the start (31 Jul) and end of thermal stress (23 Oct) and end of recovery (20 Dec). Numbers (1-2) indicate significant differences between tested groups (time*temperature*habitat) for each species according to Bonferroni pairwise tests for the GLM.

3.4.6 Caspase 3 activity

The caspase 3 activity of all control fragments did not significantly differ throughout the study (**Figure 3.6**). The caspase 3 activity of both species from both habitats in both thermal stress treatments significantly differed throughout the study (**Figure 3.6**, **Table 3.1**, and **Appendix**

14). The caspase 3 activity of both species from both habitats in both treatments was higher than their conspecifics in the control at the start of thermal stress and remained higher than the control fragments at the end of thermal stress and recovery (**Figure 3.6**, **Table 3.1**, and **Appendix 14**). At the start, the increase in caspase 3 activity in the 28°C and 30°C compared to the control was ~1823%% and ~1486% for subtidal *A. irregularis*, ~172% and ~2230% for intertidal *A. irregularis*, ~509% and ~929% for subtidal *P. verrucosa* and ~1405% and ~1431% for intertidal *P. verrucosa* (**Figure 3.6**). At the end of recovery, the elevation the caspase 3 in the 28°C and 30°C compared to the control ~25334% and ~29906% for subtidal *A. irregularis*, ~1681% and ~1790% for intertidal *A. irregularis*, ~88% and ~87% for subtidal *P. verrucosa* and ~95% and ~96% for intertidal *P. verrucosa* (**Figure 3.6**). Overall, the caspase 3 activity of *A. irregularis* and *P. verrucosa* in the 30°C treatment was ~23% and ~31% significantly higher than their conspecifics in the 28°C treatment (**Figure 3.6**, **Table 3.1**, and **Appendix 14)**. Overall, the intertidal fragments of *A. irregularis* had ~22% significantly lower caspase 3 activity than their subtidal conspecifics (**Figure 3.6**, **Table 3.1**, and **Appendix 14**). Overall, *P. verrucosa* intertidal fragments had ~19% lower caspase 3 activity although not statistically significant (**Figure 3.6**, **Table 3.1**, and **Appendix 14**). Overall, the caspase 3 activity did not differ between the two species (**Figure 3.6**).

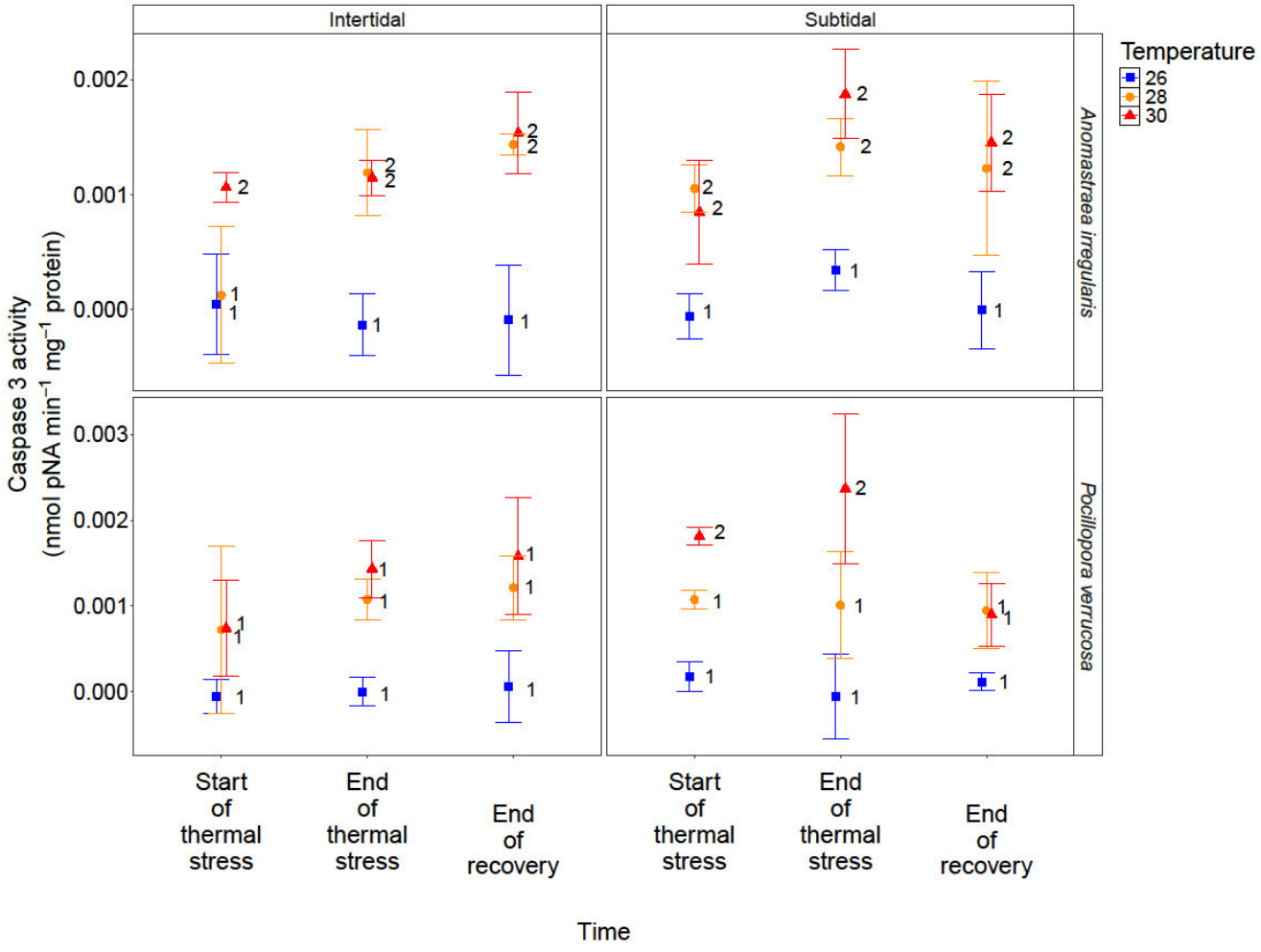

Figure 3.6: Average Caspase 3 activity (nmol pNA min^{-1} mg^{-1} of total host protein) ± 1 standard deviation in *Anomastraea irregularis* and *Pocillopora verrucosa* fragments from the intertidal and subtidal habitats at the start (31 Jul) and end of thermal stress (23 Oct) and end of recovery (20 Dec)

Table 3.1: GLM results for each species comparing the protein concentration, Superoxide dismutase activity, Catalase activity, Glutathione peroxidase activity, Glutathione S-transferase activity, and Caspase 3 activity between habitat (intertidal and subtidal), treatments (control, 28°C, and 30°C), and time (start of thermal stress, end of thermal stress, and end of recovery)

		Anomastraea irregularis			*Pocillopora verrucosa*		
Variable	Factor	Wald Chi-Square	df	*p*	Wald Chi-Square	df	*p*
Protein concentration	Time	6.089	2	0.108	4.944	2	0.084
	Treatment	17.261	2	<0.0005*	0.842	2	0.656
	Habitat	1091.163	1	<0.0005*	114.847	1	<0.0005*
	Time x Habitat	1.176	2	0.556	14.822	2	<0.0005*
	Time x treatment	6.682	4	0.154	9.469	4	0.055
	Habitat x treatment	3.503	2	0.174	25.065	2	<0.0005*
	Time x Habitat x treatment	0.656	4	0.957	13.933	4	0.075
Superoxide dismutase activity	Time	881.653	2	<0.0005*	1378.445	2	<0.0005*
	Treatment	15516.1	2	<0.0005*	27094.865	2	<0.0005*
	Habitat	839.643	1	<0.0005*	76.286	1	<0.0005*
	Time x Habitat	8.190629	2	0.0167*	12.558	2	0.002*
	Time x treatment	463.6609	4	<0.0005*	697.034	4	<0.0005*
	Habitat x treatment	406.4521	2	<0.0005*	84.55	2	<0.0005*
	Time x Habitat x treatment	14.581	4	0.006*	53.123	4	<0.0005*
Catalase activity	Time	124.8015	2	<0.0005*	353.203	2	<0.0005*
	Treatment	430.6794	2	<0.0005*	1468.743	2	<0.0005*
	Habitat	29.5955	1	<0.0005*	18.78	1	<0.0005*
	Time x Habitat	2.233961	2	0.327	0.771	2	0.68
	Time x treatment	63.86765	4	<0.0005*	169.212	4	<0.0005*
	Habitat x treatment	5.374601	2	0.068	17.701	2	<0.0005*
	Time x Habitat x treatment	4.694391	4	0.32	2.965	4	0.564

Glutathione peroxidase activity	Time	89.455	2	<0.0005*	463.108	2	<0.0005*
	Treatment	1147.462	2	<0.0005*	4046.078	2	<0.0005*
	Habitat	1.611	1	0.204	248.086	1	<0.0005*
	Time x Habitat	13.575	2	0.001*	43.572	2	<0.0005*
	Time x treatment	101.311	4	<0.0005*	422.715	4	<0.0005*
	Habitat x treatment	174.121	2	<0.0005*	257.418	2	<0.0005*
	Time x Habitat x treatment	32.258	4	<0.0005*	115.323	4	<0.0005*
Glutathione S-transferase activity	Time	0.001	2	0.998	0.155	2	0.925
	Treatment	0.001	2	0.886	63.99	2	<0.0005*
	Habitat	1.296	1	0.255	0.068	1	0.794
	Time x Habitat	0.0372	2	0.982	0.058	2	0.971
	Time x treatment	1.42	4	0.841	0.617	4	0.961
	Habitat x treatment	0.201	2	0.904	0.396	2	0.82
	Time x Habitat x treatment	0.69	4	0.953	0.137	4	0.998
Caspase 3 activity	Time	24.533	2	<0.0005*	3.322	2	0.19
	Treatment	182.72	2	<0.0005*	126.754	2	<0.0005*
	Habitat	5.917	1	0.015*	2.769	1	0.096
	Time x Habitat	7.032	2	0.03*	11.167	2	0.004*
	Time x treatment	11.518	4	0.021*	8.661	4	0.07
	Habitat x treatment	0.892	2	0.64	3.304	2	0.192
	Time x Habitat x treatment	14.46	4	0.006*	10.097	4	0.039*

3.5 Discussion

Corals are benthic sessile animals and cannot migrate when faced with stressors therefore, their adaptive biochemical cellular defence mechanisms are integral to mitigating and surviving stressors (Thummasan et al. 2021). This is the first study to investigate the long-term effects of thermal stress on the cellular defence mechanisms of *Pocillopora verrucosa* and *Anomastraea irregularis* from a high-latitude region. The protein concentration, superoxide dismutase,

catalase, glutathione peroxidase, glutathione s-transferase, and caspase 3 activities of the control fragments of both species from both habitats remained unchanged throughout the study (**Figure 3.1, 3.3, 3.4, 3.5,** and **3.6**) indicating that handling had a negligible effect on the corals and that the changes seen in the fragments in the two experimental treatments were in fact due to the thermal stress experienced. Overall, the results of this study showed that prolonged elevated temperatures caused an increase in the cellular defence mechanisms of the two species from the two habitats. Additionally, inherent biochemical and cellular differences were observed between the species and the two habitats.

Thermal stress is known to influence the protein composition in corals, including altering the expression of antioxidant proteins which can affect the overall antioxidant activity of corals (Schlöder and D'Croz 2004; Thummasan et al. 2021). Overall, the intertidal fragments of both species had higher protein concentrations than their subtidal conspecifics (**Figure 3.1, Table 3.1**). Some corals from extreme habitats may inherently maintain a higher concentration of proteins through upregulation of genes associated with protein synthesis allowing them to cope with the variable environmental conditions (Barshis et al. 2013; Schoepf et al. 2015b; Wall et al. 2018; Onyango 2020; Tisthammer et al. 2021). Higher protein concentrations in corals are known to facilitate better reaction to thermal stress at the cellular level to maintain homeostasis and resist cellular damage thereby aiding these corals to be more tolerant to thermal stress (Barshis et al. 2010; Carballo-Bolaños et al. 2019). This could explain why the protein concentrations were higher in the treatment intertidal fragments of both species compared to the controls at the start and end of the thermal stress treatments and the recovery period (**Figure 3.1**, **Table 3.1**, and **Appendix 9**). The intertidal corals may inherently maintain higher protein concentrations because of the selective environmental pressures faced and may be more efficient at increasing protein concentrations as a defence mechanism to thermal stress than the subtidal corals that don't experience the same selective pressures.

The protein concentration of the heat-stressed subtidal and intertidal *P. verrucosa* differed at different sampling points. The treatment subtidal *P. verrucosa* had significantly lower protein concentrations at the end of thermal stress and recovery than at the start of thermal stress conversely the treatment intertidal *P. verrucosa* fragments had higher protein concentration at the end of thermal stress and recovery than at the start of thermal stress (**Figure 3.1**, **Table 3.1**, and **Appendix 9**). In addition, the subtidal *P. verrucosa* fragments in the 30°C treatment had lower protein concentrations than their conspecifics in the 28°C treatment and the reverse was seen in the intertidal *P. verrucosa* (**Figure 3.1**, **Table 3.1**, and **Appendix 9**). The energy deficit

that follows bleaching can be compensated by the corals' energy reserves and proteins are utilised by some species to maintain metabolic functions (Rodrigues and Grottoli 2007). The treatment subtidal *P. verrucosa* fragments experienced more bleaching and deaths, with those in the 30°C treatment experiencing more bleaching than those in the 28°C (Chapter 2). The lower protein concentration in the heat-stressed subtidal *P. verrucosa* at the end of thermal stress and recovery than at the start of thermal stress and in those in the 30°C treatment than those in the 28°C treatment could therefore be because the corals used the proteins as an energy source and/or the corals were not able to upregulate proteins. The higher protein concentration in the treatment intertidal *P. verrucosa* fragments at the end of thermal stress and recovery than at the start of thermal stress and in the 30°C treatment than those in the 28°C treatment may be because the fragments were able to upregulate protective proteins as a defence mechanism. Upregulation in genes associated with protein synthesis in corals from extreme habitats when heat-stressed can lead to higher protein concentrations (Onyango 2020; Onyango et al. 2021).

The present study also found innate differences in the protein concentrations between the two species, where the massive species had higher protein concentrations than the branching species (**Figure 3.1**). This corresponds with the transcriptome findings of Onyango (2020) where *A. irregularis* had higher expression of genes associated with its metabolism and protein synthesis at baseline and significantly upregulated them as a protective mechanism when exposed to 24 hours of heat stress compared to *P. verrucosa*. It is possible that *A. irregularis* can maintain the upregulation of these genes during prolonged thermal stress, as the present study showed. This may also explain why the protein concentrations in the treatment intertidal and subtidal *A. irregularis* did not differ at the different sampling points and between the temperature treatments. The present study is supported by previous work, which has similarly found slow-growing massive coral species to have higher protein concentrations and to be more resilient to thermal stress than fast-growing branching corals (Schlöder and D'Croz 2004).

Both species from both habitats in both temperature treatments had significantly higher superoxide dismutase activity than the controls at the start of the experiment (**Figure 3.2**, **Table 3.1**, and **Appendix 10**). This enzyme is the first line of defence against reactive oxygen species and has been well documented to increase activity in corals exposed to thermal stress (Downs et al. 2002; Lesser 2004; Flores-Ramirez and Linan-Cabello 2007; Fitt et al. 2009; Weis 2010; Dias et al. 2019a; Dias et al. 2019b; Huang et al. 2024). The accessory antioxidant enzymes catalase and glutathione peroxidase activity in both species from both habitats in both thermal stress treatments were also significantly higher than their conspecifics in the control at the start

of thermal stress (**Figure 3.3**, **Figure 3.4**, **Table 3.1**, **Appendix 4**, and **Appendix 12**). Coral hosts have also been found to increase the activity of these two enzymes to scavenge hydrogen peroxide at the onset of thermal stress before photosynthetic dysfunction and bleaching can occur (Yakovleva et al. 2004; Krueger et al. 2015; Dias et al. 2019a; Dias et al. 2019b; Huang et al. 2024). The activity of the three enzymes decreased at the end of the thermal stress and recovery period but remained significantly higher than the control fragments (**Figure 3.2**, **3.3**, **3.4**, **Table 3.1**, **Appendix 3**, **Appendix 4**, and **Appendix 12**). The elevated activity of these enzymes in response to thermal stress was usually considered to be short-term and unsustainable during prolonged stress periods (Griffin et al. 2006). However, Dias et al. (2019a) also found elevated antioxidant enzyme activity in some coral species exposed to thermal stress for 60 days. The prolonged elevated activity of these enzymes suggests that the antioxidant enzyme production systems were not completely overwhelmed or exhausted during this study (Dias et al. 2019a). Furthermore, the elevated activity after the recovery period showed that the biochemical systems of the corals were still stressed and did not reach homeostasis suggesting that these corals require a longer recovery period to reach full biochemical and physiological recovery. This could affect the reef trajectories *in situ* since recovery time will be decreased with the increased frequency and intensity of bleaching events predicted (Hoegh-Guldberg et al. 2023). The long-term cost of maintaining elevated antioxidant activities may lead to lower fitness and may compromise the corals to diseases (Pinzón et al. 2014) and therefore warrants further investigation.

The control fragments appeared to have negligible superoxide dismutase and glutathione peroxidase activity, and there were no apparent differences between the species and habitats (**Figure 3.2**, **3.4**, **Table 1**, **Appendix 10**, and **Appendix 12**). However, the catalase activity of the subtidal control fragments of both species was noticeably higher than the intertidal control conspecifics (**Figure 3.3**, **Table 3.1**, and **Appendix 11**). This could have been because catalase activity can be a product of metabolism (Downs et al. 2012), and the subtidal corals were found to have higher respiration rates (Chapter 2). Furthermore, thermal stress, induced noticeable differences in superoxide dismutase, catalase, and glutathione peroxidase activity between habitats and species. For both species, the intertidal fragments in thermal stress treatments had significantly lower superoxide dismutase, catalase, and glutathione peroxidase activity than their subtidal conspecifics (**Figure 3.2**, **3.3**, **3.4**, **Table 3.1**, **Appendix 10**, **11**, **12**). In the two thermal stress treatments the superoxide dismutase, catalase, and glutathione peroxidase activity of *A. irregularis* also appeared lower than the *P. verrucosa* fragments (**Figure 3.2**, **3.3**,

3.4, **Table 3.1**, **Appendix 10, 11, 12**). The subtidal corals and *P. verrucosa* were seen to be less resilient to thermal stress (Chapter 2), and it is thought that the higher antioxidant activities in these corals may have resulted from higher oxidative stress experienced due to their susceptibility to thermal stress. This is in congruence with a short-term heat stress study that found only 1.23% of the more resilient *A. irregularis* transcriptome was affected with average low fold changes in individual genes while 33.58% of the transcriptome of the susceptible *P. verrucosa* was affected with larger fold changes of up to 10.2 in individual genes (Onyango 2020). Other studies have similarly found sensitive corals to have increased activity of antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase with increased thermal stress because these corals have a lower reactive oxygen species threshold and, therefore would rapidly increase their antioxidant activity to counteract the oxidative stress (Downs et al. 2002; Griffin et al. 2006; Krueger et al. 2015; Dias et al. 2019a; Huang et al. 2024). However, some studies have shown that resilient corals from environmentally variable habitats or those that experienced repeat thermal stress events have higher antioxidant activity that makes these corals better equipped to neutralise oxidative stress (Flores-Ramirez and Linan-Cabello 2007; Fitt et al. 2009; Tisthammer et al. 2021; Wang et al. 2022; Zhai et al. 2024). This highlights the variability in antioxidant activity in corals and the need to investigate it in coral species from diverse locations and environmental regimes. The variability in the antioxidant activity of corals also shows that investigating the activity alone is not universally predictive of bleaching trajectories, and therefore, other physiological parameters need to be investigated (Dias et al. 2020).

Glutathione s-transferase activity did not significantly differ between habitats nor between species (**Figure 3.5**, **Table 3.1**, and **Appendix 13)**. This enzyme is important for detoxifying xenobiotics (Downs et al. 2012) and, therefore, may not have been upregulated in this controlled laboratory study. This enzyme may be more important *in situ* where corals have a higher chance of encountering pollution (Anithajothi et al. 2014). This enzyme can also detoxify lipid peroxides (Limon-Pacheco and Gonsebatt 2009). Both thermal treatments induced significantly higher levels of the enzyme activity, highlighting that there may have been lipid peroxidation occurring in the thermally stressed corals. However, the enzyme activity was negative, so it could be that these corals did not have high enough levels of lipid peroxides present to warrant upregulation of the enzyme or that other enzyme pathways covered the detoxification. Therefore, based on the results, this particular enzyme may not play an integral part in the resilience of prolonged thermal stress.

Consistent with literature (Ainsworth et al. 2008; Kvitt et al. 2016; Yu et al. 2017; Thummasan et al. 2021), the present study found the caspase 3 activity of both species from both habitats in both thermal treatments was significantly higher than their conspecifics in the control (**Figure 3.6**, **Table 3.1**, and **Appendix 14**). Furthermore, the caspase 3 activity of both species from both habitats in the 30°C treatment was significantly higher than their conspecifics in the 28°C treatment (**Figure 3.6**, **Table 3.1**, and **Appendix 14**). Excess reactive oxygen species overwhelms the antioxidant defence mechanisms damaging DNA, protein, and cell membranes, leading to apoptosis and tissue necrosis (Lesser 1997; Thummasan et al. 2021). The present study indicates that despite the increased antioxidant activities in the treatment corals (**Figure 3.2**, **3.3**, **3.4**, **Table 3.1**, **Appendix 10**, **11**, **12**), the temperature treatments had caused enough stress to the corals to initiate apoptosis and that the higher (30°C) temperature treatment understandably caused more programmed cell death (**Figure 3.6**, **Table 3.1**, and **Appendix 14**). Overall, lower caspase 3 activity was found in the intertidal fragments of both species than their subtidal conspecifics (**Figure 3.6**, **Table 3.1**, and **Appendix 14**). This correlates with the differences in the antioxidant activities found between the habitats. It appears that the intertidal corals had lower caspase 3 activity (**Figure 3.6**, **Table 3.1**, and **Appendix 14**) since they experienced less oxidative stress as seen with the lower antioxidant enzyme activities (**Figure 3.2**, **3.3**, **3.4**, **Table 3.1**, **Appendix 10**, **11**, **12**) less bleaching and mortalities (Chapter 2) while the subtidal corals that had higher antioxidant enzyme activity (**Figure 3.2**, **3.3**, **3.4**, **Table 3.1**, **Appendix 10**, **11**, **12**) had higher caspase 3 activity (**Figure 3.6**, **Table 3.1**, and **Appendix 14**) and experienced more bleaching and mortalities (Chapter 2). Surprisingly, though, the caspase 3 activity did not noticeably differ between the two species as the trend with the antioxidant activities (**Figure 3.6**). The caspase 3 enzyme is not the only protease involved in apoptosis, but there are other effector proteases like caspase 6 and caspase 7 (Thornberry et al. 1997) that may shed further light on apoptotic events in these treatments. The caspase 3 activity remained significantly higher than the control fragments at the end of thermal stress and recovery (**Figure 3.6**, **Table 3.1**, and **Appendix 14**). This coincides with the antioxidant enzyme activities and further indicates that the corals were still stressed and were not completely recovered.

The corals in the 30°C treatment would have been exposed to eight days of increasing temperatures while those in the 28°C treatment would have been exposed to four days. It is noteworthy that in the short amount of time a strong molecular response was mounted since most antioxidant enzymes studied here were significantly elevated at the start of heat stress

(when target treatment temperatures were reached). Furthermore, the enzyme activities were not fully back to control levels after two months of recovery. The prolonged upregulation of enzymatic activities would indeed have energetic implications for the corals. Lipids can be used as a source of energy when thermally stressed (Rodrigues and Grottoli 2007). In Chapter 2 it was evident that the thermally stressed corals had severely depleted lipid contents at the end of the heat stress and even after the recovery period (**Figure 2.5d**). This shows that mounting a strong and prolonged enzymatic response to thermal stress is costly. A limitation of the present static laboratory study is that temperature was the only variable considered. Under natural conditions, other environmental variables also affect the antioxidant enzyme activity of corals (Anithajothi et al. 2014). Light stress in particular plays a significant role in coral bleaching and the upregulation of antioxidant enzyme activity and heat shock proteins (Desalvo et al. 2008; Hawkins et al. 2015; Rosic et al. 2020; Thummasan et al. 2021). The average PAR in this study was much lower than what shallow-water corals *in situ* usually experience (Dubinsky et al. 1990; Lehmann 2022). The PAR level was chosen in this study so that the subtidal and intertidal corals could share the tanks, and the levels could still be considered environmentally relevant during lower light conditions experienced with weather and pollution conditions. However, the lower light could have mitigated some of the thermal stress experienced by the corals since reduced light intensity has been found to ameliorate thermal stress effects in some corals (Desalvo et al. 2008; Hawkins et al. 2015; Rosic et al. 2020; Thummasan et al. 2021).The coral host is integral in the health and persistence of coral reefs under thermal stress (Bellantuono et al. 2012b; Krueger et al. 2015; Drury 2020; Wang et al. 2022; Huang et al. 2024). However, it is noteworthy that the Symbiodiniaceae also possess these antioxidant enzymes to deal with reactive oxygen species (Krueger et al. 2015; Higuchi 2018; Huang et al. 2024). The antioxidant activities of the coral hosts were only investigated in the present study as it was believed that the hosts were more responsible for the differential responses to thermal stress since Onyango (2020) found no genetic differences in the Symbiodiniaceae clade housed by *P. verrucosa* and *A. irregularis* from the intertidal and subtidal habitats and there was little change in the Symbiodiniaceae transcriptomes from both species when exposed to short-term heat stress. However, future studies should investigate the role of the Symbiodiniaceae antioxidant capacity in the thermal resilience of the coral holobionts.

Ultimately, the present study found that both temperature treatments caused elevated antioxidant enzyme activities in both species from both habitats, with the 30°C treatment being

more detrimental to the corals causing more caspase 3 activity than the 28°C treatment. This highlights that even the conservatively predicted increase in temperature can impact the biochemical cellular defences of these corals. The results correspond with the oxidative bleaching theory since the higher antioxidant enzyme activities suggests that the corals experienced higher oxidative stress with the thermal stress treatments. Moreover, the present study found differences in the enzyme activities between species and habitats when thermally stressed. The hypothesis that intertidal corals and *A. irregularis* would have higher baseline antioxidant enzyme activity was not supported, as they exhibited no difference in baseline enzyme activity but higher protein concentrations. However, the hypothesis that antioxidant enzyme activity would increase under thermal stress was supported. Higher protein concentrations and lower superoxide dismutase, catalase, and glutathione peroxidase activity were found in the thermally resilient intertidal fragments than subtidal fragments and *A. irregularis* than *P. verrucosa* when thermally stressed. These corals could have been more resilient to prolonged thermal stress because the higher protein concentration would have allowed for favourable physiological processes that lowered oxidative stress to a manageable threshold, thereby not requiring as much of an increase in antioxidant activities as the susceptible corals. Contrary to the hypothesised decrease in enzyme activity at the end of recovery, two months of recovery was not enough for these corals to return to baseline antioxidant activity, therefore, the long-term effects of maintaining higher antioxidant activities need to be investigated considering near-future climate change predictions.

3.6 Conclusion

The antioxidant capabilities of corals can influence their susceptibility/resilience to thermal stress and, therefore, need to be investigated in species from extreme and marginal habitats to aid in coral reef conservation initiatives. Overall, prolonged thermal stress caused long-term increased oxidative stress, as seen with the increased superoxide dismutase, catalase, glutathione peroxidase, and caspase 3 activity in fragments in both thermal stress treatments at the start and end of thermal stress and at the end of recovery. The results also showed inherent differences between habitat and species highlighting that environmental regimes and species may influence the antioxidant potential of corals. Overall, higher protein concentrations and lower antioxidant enzyme activities (superoxide dismutase, catalase, and glutathione peroxidase) were evident in intertidal fragments than subtidal fragments and *Anomastraea irregularis* than *Pocillopora verrucosa* when thermally stressed. The higher protein concentrations may have facilitated physiological processes in these corals that made them

more resilient to prolonged thermal stress. Therefore, these corals may have experienced less oxidative stress and, therefore, required less antioxidant activities. The results indicated much higher antioxidant activity in susceptible corals thereby suggesting that high oxidative stress may be responsible for higher bleaching and mortalities. Two months of recovery was not enough for these corals to return to baseline antioxidant activity, therefore, the long-term effects of maintaining higher antioxidant activities need to be investigated considering near-future climate change predictions.

Chapter 4: Flexi-chambers and 3D photogrammetry effectively measure coral fragment physiology *in situ*

4.1 Abstract

Non-invasive techniques for measuring the physiological performance of corals *in situ* are important for understanding responses to changing environmental conditions. Two such methods are the 'flexi-chamber' and three-dimensional (3D) photogrammetry. While these have been verified for coral colonies, they have not been tested on coral fragments which are commonly used in field experiments. This study aimed to determine if these methods can effectively measure respiration, photosynthesis, and growth of fragments of massive and branching coral species in the intertidal and subtidal habitats in Park Rynie, South Africa (30.3167° S, 30.7333° E). Differences in respiration and photosynthesis rates between species and habitat were successfully measured using the flexi-chambers. The coral fragments did not show any visual signs of stress (excess mucus production or loss of pigmentation) during the 3-hour incubations or an hour after the incubations. 3D models were successfully constructed for all fragments. This study showed that flexi-chambers and 3D photogrammetry can be effectively used to measure the respiration, photosynthesis, and growth of individual coral fragments (3 cm > 4 cm) of different morphology and habitats. The combination of the two methods permits measures of fundamental physiological processes to be attained at relatively low cost and low complexity. This will facilitate studies of a wide range of coral species *in situ* generating more knowledge on responses to environmental variability.

Keywords: respiration, photosynthesis, growth, coral fragment, photogrammetry

4.2 Introduction

Reef-building corals which are integral to reef ecosystems have been declining at an unprecedented rate in recent decades due to several anthropogenic activities (Hughes et al. 2003; Hoegh-Guldberg et al. 2007; Glassom 2014; Hoegh-Guldberg et al. 2018; Kubicek et al. 2019). Coral reefs are experiencing increased global mass bleaching events due to ocean warming (Hughes et al. 2017b; Hughes et al. 2018a; Raymundo et al. 2019). The extensive loss of corals leads to a decline in habitat complexity and therefore a drastic loss in productivity in both an ecological and socio-economic sense (Moberg and Folke 1999; Alvarez-Filip et al. 2011; Alvarez-Filip et al. 2013; Dietzel et al. 2021).

Vital physiological processes like respiration, photosynthesis, and growth give indications of the coral's performance under varying environmental conditions (Coles and Jokiel 1977; Porter et al. 1999; Lesser 2013). These processes in corals also form the basis of reef ecosystems through regulating biogeochemical nutrient cycles and complex ecological interactions, and therefore inform effective reef management (Langdon and Atkinson 2005; Junjie et al. 2014). Determining the baseline physiological rates of reef-building corals and when they face environmental changes can also provide a foundation for understanding how future environmental perturbations may affect corals (Wall et al. 2021). However, measuring the rates at which these processes occur has proven to be quite challenging in the past since it typically involved using fairly expensive and cumbersome *in situ* respirometry chambers (Patterson et al. 1991; Yates and Halley 2003; Levy et al. 2004; Nakamura and Nakamori 2009; Sawall et al. 2011; Murphy et al. 2012; Dove et al. 2013; Okazaki et al. 2013). In addition, corals of interest usually had to be removed and analysed *ex situ* which limits the scale, resolution, and the accuracy of the measurement of metabolic rates (Rex et al. 1995; Nystrom et al. 2001; Naumann et al. 2013). The flow rates around the corals are also drastically reduced when using rigid sealed glass or plexiglass respirometry chambers which prevents the disruption of the diffuse boundary layer around the surface of the corals. Therefore, artificial mixing must be employed in the chambers through stir bars and magnets (Davies 1980; Telesnicki and Goldberg 1995), marbles (Sawall et al. 2011) or semi-continuous automatic flushing systems (McCloskey et al. 1978; Kegler et al. 2015), which leads to increased technical complexity and sampling effort (Camp et al. 2015). A gas-impermeable plastic bag with a built-in, heat-seam secured valve called the 'flexi-chamber' was developed by Camp et al. (2015) to improve metabolic rate analyses of corals *in situ* by mitigating the many constraints of the traditional rigid glass and plexiglass incubation chambers. This method is more cost-effective and allows

researchers to increase their sampling scale without needing to remove corals from the field (Camp et al. 2015). The flexible nature of the bag allows for the manipulation of the bag around colonies of different morphologies thereby adjusting the volume of water surrounding the colonies during incubation, and thus minimising potential toxicity from supersaturation of oxygen or anoxic conditions (Camp et al. 2015). The valve allows for the easy removal of sample water to detect changes in the oxygen levels and to calculate calcification rates of corals during incubations.

Coral growth has been measured in several different ways such as the change in mass, volume, area, or linear extension per unit time (Buddemeier and Kinzie III 1976; Jokiel et al. 1978; Davies 1989; Holcomb et al. 2013; Fantazzini et al. 2015; Lizcano-Sandoval et al. 2018). No single method to measure coral growth has been universally adopted due to difficulties arising from the complex shapes of coral colonies and variable growth regimes for individual species (Buddemeier and Kinzie III 1976; Veal et al. 2010; Herler and Dirnwöber 2011). Several methods are damaging (Dodge et al. 1984) and invasive to coral colonies while others require removing the colonies from the substrate and using an expensive and heavy mass balance to weigh the colonies (Davies 1990). The analysis of seawater via titration to determine the calcification rates of colonies also involves using expensive equipment and reagents (Camp et al. 2015). In contrast, three-dimensional (3D) photogrammetry is a fairly inexpensive, non-invasive alternative to measure coral growth accurately as it takes into account the structural complexity across different coral morphologies (Figueira et al. 2015; Lavy et al. 2015; Ferrari et al. 2016; Guo et al. 2016; Ferrari et al. 2017; Lange and Perry 2020). Photogrammetry can give measures of the change in volume and surface area of the coral colony which are important measures of interest since they influence the structure and functioning of the reef ecosystems (Ferrari et al. 2017). The 3D photographic method requires a submersible camera, a computer with 3D software for processing images or video, and processing time (Figueira et al. 2015). With advances in technology the process has become less costly and more user friendly (Lange and Perry 2020).

Though the 3D photogrammetry and flexi-chamber methods have been proven to provide reliable results using coral colonies these techniques have not been tested for use with coral fragments < 4 cm *in situ*. Coral fragments allow for more replicate measurements across several treatments with the same amount of source material (Shafir et al. 2003; Shafir et al. 2006). Furthermore, the use of fragments from the same colony in each treatment removes the possibility that genetic variability, rather than the treatment, is responsible for any differences

in responses. Using fragments from at least four colonies in experiments yields a larger understanding of the population responses to stressors without negatively impacting the already declining populations of corals (Baums et al. 2019; McLachlan et al. 2020). Since it is important to use fragments in physiological assays this study investigated the viability of the flexi-chambers and 3D photogrammetry to measure respiration, photosynthesis, and growth of two species of different morphologies and habitats *in situ*. The combination of these cost-effective and relatively simple methods could provide insight into how corals respond to changing conditions.

4.3 Methodology

4.3.1 Collection of coral fragments

Anomastraea irregularis was chosen for use in experiments as it is a common massive coral and *Pocillopora verrucosa* was chosen as it is a common branching coral on the South eastern coast of KwaZulu-Natal, South Africa (Smit and Glassom 2017). The sampling was conducted during December 2021-January 2022 (see **Appendix 15** for flow chart) under the Department of Environment, Forestry and Fisheries permit RES2021-38. Ten healthy *A. irregularis* (3.04 ± 0.36 cm) and ten healthy *P. verrucosa* (3.08 ± 0.41 cm) fragments were taken randomly from ten colonies in the intertidal and the subtidal habitats (**Appendix 16**) at Park Rynie (30.3167° S, 30.7333° E) on the South Coast of KwaZulu-Natal, South Africa. Coral fragments were considered healthy if they had no visual signs of disease and fell into category 5-6 on the Coral Health Chart (Coral Watch, Australia). The *A. irregularis* fragments were collected using a hammer and chisel while the *P. verrucosa* fragments were collected using wire cutters. Each fragment was fixed onto a concrete disk (9 cm diameter and 2 cm thick) using epoxy putty (Pratley® Quickset Putty, South Africa). The concrete disks containing fragments were placed back into the intertidal and subtidal habitats using epoxy putty. The fragments were randomly distributed in both habitats with approximately half a meter of spacing between each other. The fragments were left to recover from sampling for a week before measurements were taken. In addition, five concrete disks without any coral fragments were placed in the intertidal habitat and another five were placed in the subtidal habitat.

4.3.2 Flexi-chamber method description

4.3.2.1 Chamber description

The flexi-chamber method was adapted from Camp et al. (2015). The flexi-chambers used in this study were 2-L transparent gas-impermeable flexible urine bags with built-in heat-seamed secured valves (Glomed, India) (**Figure 4.1 a** and **b**). The bottom of each bag was cut to create

an opening. A soft flexible rubber (Industrial rubber suppliers, South Africa) was attached to the inside of the opening (**Figure 4.1 c**) to create a watertight seal around the concrete base (**Figure 4.1 d**). The concrete base also reduced potential contribution of the surrounding substrate and/or water column to the metabolic signal of the coral fragments. The bag was fastened to the concrete base using two cable ties (4.8 x 400 mm).

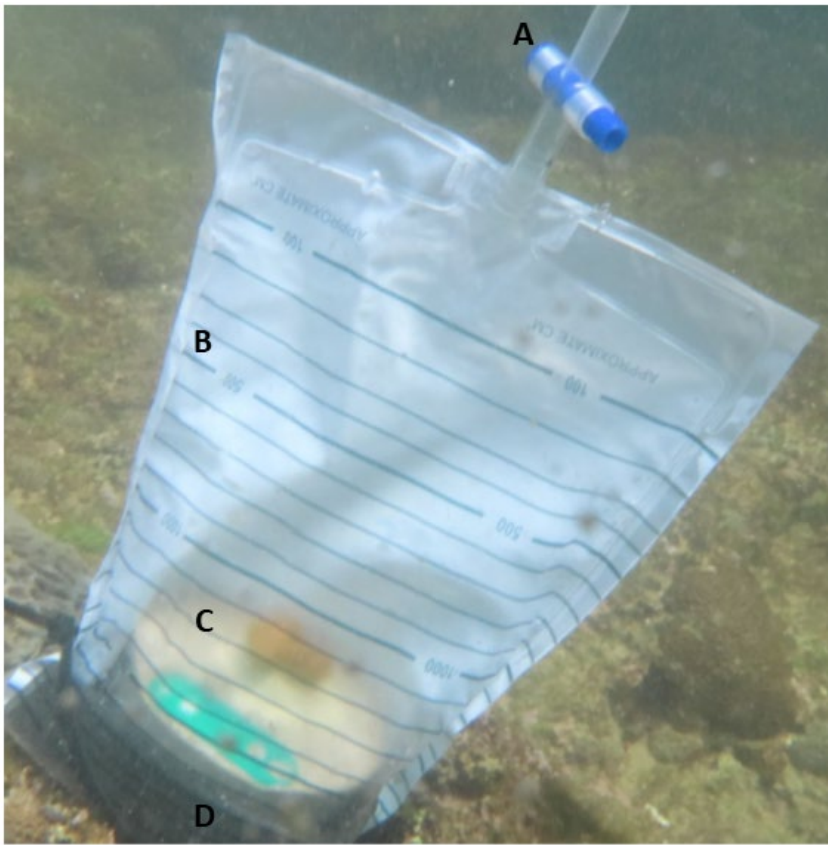

Figure 4.1: Flexi-chamber deployed *in situ* consisting of **A** three-way valve, **B** 2-L urine bag, **C** the concrete disk base with green plastic identification tag, **D** black flexible rubber stuck on the inside opening of the bag

4.3.2.2 Chamber validation

To determine if the connection was indeed watertight, 60 mL of natural red food colouring was syringed into the ten blank chambers (concrete disks without corals) through the valve (Mallon et al. 2022). The chambers and surrounding water were monitored visually by two people immediately after insertion of the colour and then every 15 minutes for 3 hours to detect any leakage of colour. During the incubation, an Onset HOBO Pendant Temperature/Light (model UA-002-64; Microdaq, United States of America) (set to log every 30 seconds) and a G Pendant acceleration logger (UA-004-64; Microdaq, United States of America) (set to log every second) were placed inside the chambers. The G Pendant logger gave measurements of water

movement in the X, Y, and Z plane. The same type of loggers were also placed outside the chambers to determine if the temperature, light and water movement inside the chambers were significantly different to the outside conditions (Camp et al. 2015). To determine if the flexi-chambers reduced the transmission of photosynthetic active radiation (PAR), three flexi-chambers were placed in a 160-L aquarium tank equipped with a LED aquarium light (ZT6600II; Zetlight, China) at the Marine Science Unit (MSU) at the University of KwaZulu-Natal, South Africa. The PAR inside the flexi-chambers and directly outside of the chambers were then measured using a PAR meter (MQ-510; Apogee, United States of America).

4.3.2.3 Determination of respiration and photosynthetic rates of coral fragments

The chambers were acid washed with 2% hydrochloric acid (Associated Chemical Enterprises, South Africa) before every experimental use (Camp et al. 2015). The photosynthetic rates were measured using 3-hour incubations during the day 11:00-14:00. The experimental control comprised five chambers attached to the concrete disks without any coral fragments. The control chambers were used to correct for any metabolic activity within the surrounding water not due to the corals. Two prelabelled 60-mL syringes (Healthease, Neomed, South Africa) were used for each chamber so that a total of 120 mL of seawater was removed from each chamber through the built-in valve mechanism using syringes. The seawater syringed from each chamber at the start of the incubation period and at the end (3 hours later) was immediately transferred to prelabelled 250-mL glass bottles (Camp et al. 2015). The dissolved oxygen concentrations were measured in the field immediately after the transfer using a portable dissolved oxygen meter (T-Heng M1500K, China). The chambers were removed after 3 hours and flushed with ambient water (Camp et al. 2015). They were then reattached and covered with black plastic bags, each held in place by three cable ties, to induce dark cycle metabolism (McCloskey et al. 1978; Camp et al. 2015). The removal of seawater was also done at the start and 3 hours later at the end of the dark cycle. Preliminary trials indicated that the duration and volume of the chamber used for the coral incubations were suitable to detect a maximum of 30% increase/decrease of the initial oxygen concentration thereby avoiding harmful oxygen concentrations (Biscere et al. 2019; Nelson and Altieri 2019).

The gross photosynthetic and respiration rates were determined using the equation following Camp et al. (2015):

$$Gross\ photosynthetic\ and\ respiration\ rates\ = \left[\frac{(\Delta O_2) \times V}{I_t \times SA}\right]$$

Where the change in the oxygen concentrations (ΔO_2) during the dark incubations represented the dark respiration rates and the changes during the light incubations represented the combination of respiration and photosynthesis. Gross photosynthetic rates were obtained by subtracting the oxygen flux during dark incubation (dark respiration) from the oxygen flux during the daylight incubation (net photosynthesis + dark respiration), assuming the same respiration rates during light and dark phases (McCloskey et al. 1978; Camp et al. 2015). The respiration rate values were subsequently converted to positive values by multiplying the values by the factor -1 (McCloskey and Muscatine 1984; Camp et al. 2015). The volume of water (V) surrounding the coral within the chamber was determined by syringing the water out through the valve after the incubation time (Camp et al. 2015). The volume of the coral fragments was attained using the 3D photogrammetry method described below. The respiration and photosynthetic rates were normalised by the fragment surface area (SA) calculated using the 3D photogrammetry method described below, and I_t accounted for the incubation time in hours.

4.3.3 3D photogrammetry

The 3D photogrammetry method was adapted from Lange and Perry (2020). Short 2-3-minute videos (Camera video settings: 4K resolution, f 5.6, ISO 1/100, no flash, no zoom, landscape orientation) were taken of each fragment using a bridge camera (Canon PowerShot SX70 HS, Japan) enclosed in a custom underwater housing with a flat lens port. A 30-cm ruler was placed on two sides of each fragment before taking videos to function as a reference for scaling the resulting 3D models. Strips of the Coral Health Chart (Coral Watch, Australia) were stuck onto the centre of each ruler and an 800-g dive weight was attached under each ruler to prevent it from moving during video acquisition. Each fragment was videoed from all angles following a pattern described by Lange and Perry (2020) to ensure that still images with a 70-80 % overlap between pictures could be obtained. Sixteen small objects (6 wood rectangles, 1 wood triangle, 2 wood half cylinders, 2 wood cylinders, 2 wood cubes, 3 plastic cylinders) were stuck near the coral fragments in the intertidal habitat and 15 small objects, as above but with only 2 plastic cylinders were stuck near the coral fragments in the subtidal habitat. These objects were videoed in the same manner as the coral fragments to determine the accuracy of the surface area measurements attained from the 3D software as opposed to physical measurements of the objects attained using Vernier callipers.

Still images were obtained for each video using the scene video filter tool in the software VLC media player (VideoLAN 3.0.16 Ventinari). Every 20th image was saved for each video

resulting in approximately 240-360 images per video. The images were imported into Agisoft Metashape (Version 1.7.2 build 12070 / 18 March 2021) which was used to construct the 3D models of the fragments and objects. The workflow to construct the 3D models and surface area measurements followed Lange and Perry (2020). The volume of the coral fragments were also obtained following Lange and Perry (2020) to be used in the calculation of metabolic rates mentioned above.

To compare the surface area measurements attained from 3D photogrammetry to measurements attained using the typically used wax dip method (Stimson 1991), the surface area of fourteen rectangular wooden blocks were measured using 3D photogrammetry, Vernier callipers as described above, and then the wax dip method as described by Veal et al. (2010). The surface area of ten *A. irregularis* and ten *P. verrucosa* (**Appendix 17**) taken from the intertidal habitat of Park Rynie were also measured using the 3D photogrammetry method and then the wax dip method.

4.3.4 Statistical analyses

The assumption of normality was violated even after log data transformation therefore Mann-Whitney U tests were performed to determine if the temperature, light intensity, and water movement was significantly different inside the chamber compared to outside the chamber. An independent samples t-test was done to determine if PAR significantly differed inside the chamber compared to outside the chamber. A two-way Analysis of Variance (ANOVA) was conducted to determine significant differences in the respiration and photosynthetic rate between species and habitat. The assumptions of normality and equal variances of studentised residuals were satisfied (one sample Kolmogorov-Smirnov on residuals of the dependent variables $p>0.05$ and ANOVA on residuals of the dependent variables $p>0.05$). The interaction term of species*habitat was not significant for respiration rates, photosynthetic rates, and surface area of coral fragments therefore the ANOVA analyses were run again without the interaction term. However, Bonferroni pairwise comparisons using estimated marginal means were conducted to further show any statistical differences between species*habitat.

A Wilcoxon Signed Ranks test was done to determine if there was a significant difference in the surface area of the wooden and plastic objects measured physically with a calliper and using the 3D software. Non-parametric tests were performed because the data were found to not fit a normal distribution (one sample Kolmogorov-Smirnov $p<0.05$), log data transformation also did not satisfy the assumption of normality. A two-way ANOVA was conducted to determine

if there was a significant difference in the surface area of fragments between the two species and habitats. The assumptions of normality and equal variances of studentised residuals were satisfied (one sample Kolmogorov-Smirnov on residuals of the dependent variable p=0.227 and ANOVA on residuals of the dependent variable p=1.000).

A related samples Friedman's test was done to compare the surface area measurements of the rectangular wooden blocks attained using the three different methods. The assumption that the residuals are normal (one sample Kolmogorov-Smirnov on residuals of the dependent variable $p<0.05$) was not satisfied even after log data transformation therefore the non-parametric test was done. A paired-samples t-test was done for each species to determine if the surface area measurements attained using the wax dip method were significantly different to the measurements attained from the 3D photogrammetry method. The assumption of normality was satisfied (one sample Kolmogorov-Smirnov $p>0.05$ in both instances).

4.4 Results

4.4.1 Flexi-chamber

No leakage of colour was witnessed after monitoring the chambers and surrounding seawater. There were no significant differences in temperature (**Figure 4.2, Table 4.1**), light intensity (**Figure 4.2, Table 4.1**), or acceleration (X, Y, Z planes) (**Figure 4.2, Table 4.1**) between the inside and outside of the chamber during the 3 h incubation in either habitat. The independent samples t-test (t=-1.982, df=16, p=0.065) indicated that the PAR inside and outside the chambers were not significantly different (**Figure 4.2**). The coral fragments did not show any visual signs of stress (excess mucus production or loss of pigmentation) during the 3-hour incubations and an hour after the incubations. The respiration rates of the coral fragments significantly differed between species and habitats (**Figure 4.3, Table 4.2**), however the Bonferroni pairwise comparisons showed that only *A. irregularis* respiration rates significantly differed between habitats and not *P. verrucosa* (**Appendix 18**). The photosynthetic rates of the coral fragments significantly differed between species but not between habitats (**Figure 4.3, Table 4.2, Appendix 18**).

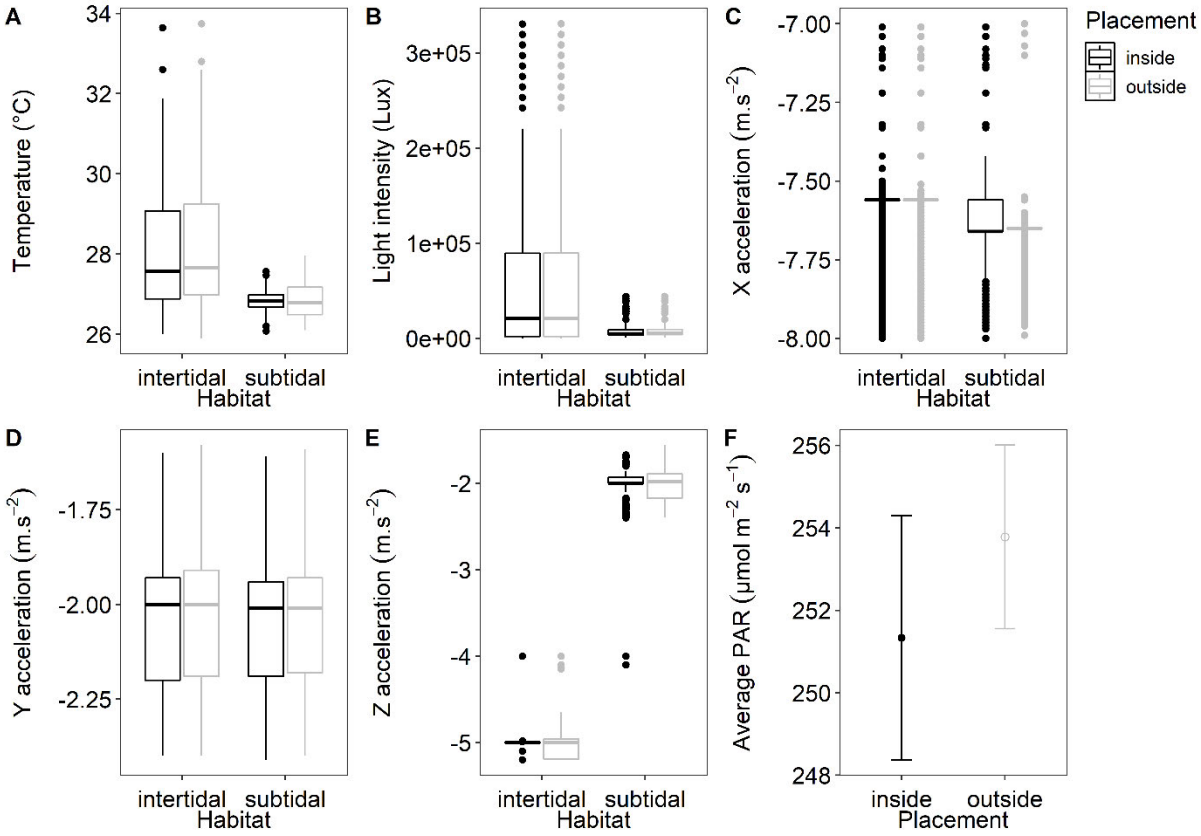

Figure 4.2: **A** The temperature (°C), **B** the light intensity (Lux), and the acceleration (m.s^{-2}) in the **C** X, **D** Y, **E** Z planes inside and outside of the chambers in the intertidal and subtidal habitat during 3 hours of incubation (11:00-14:00), **F** average (±1SD) PAR measured inside and directly outside the flexi-chamber bags at the MSU

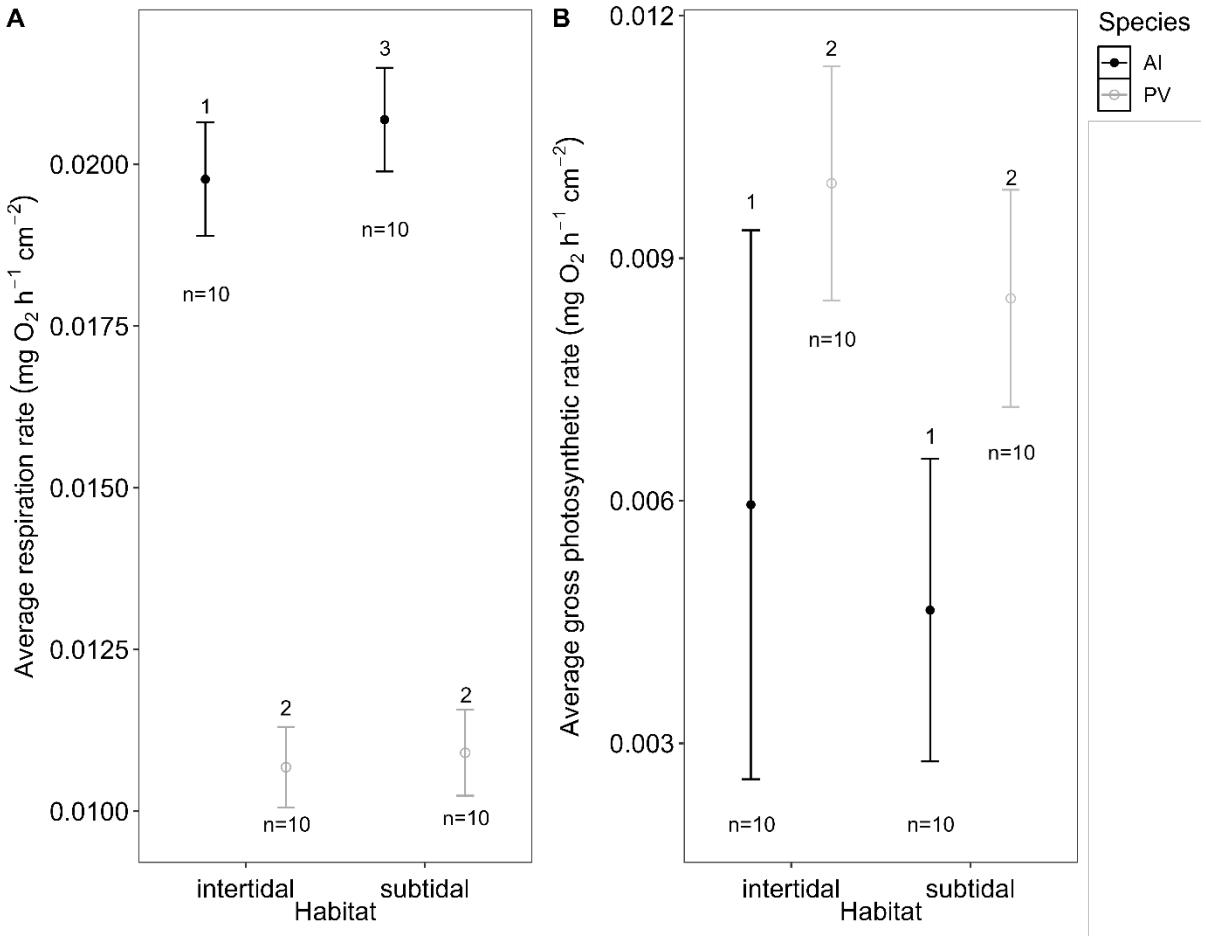

Figure 4.3: A The average (±1SD) respiration rate (mg O_2 h^{-1} cm^{-2}) of *Anomastraea irregularis* (AI) and *Pocillopora verrucosa* (PV) in the intertidal and subtidal habitats **B** the average (±1SD) gross photosynthetic rate (mg O_2 h^{-1} cm^{-2}) of *Anomastraea irregularis* (AI) and *Pocillopora verrucosa* (PV) in the intertidal and subtidal habitats. Numbers (1-3) indicate the Bonferroni pairwise differences (**Appendix 18**)

Table 4.1: Results of the Mann-Whitney U tests for comparing temperature (°C), light intensity (Lux) and water movement ($m.s^{-2}$) inside and outside the chambers in the intertidal and subtidal habitats over 3 hours

	Intertidal			Subtidal		
Physical parameter	Mann-Whitney U	n	*p*	Mann-Whitney U	n	*p*
Temperature	68236.5	720	0.218	66871.5	720	0.457
Light intensity	63705	720	0.695	62651	720	0.441
X plane acceleration	57769326.5	21600	0.149	57597231	21600	0.098
Y plane acceleration	57440240.5	21600	0.055	57954624.5	21600	0.425
Z plane acceleration	57683406.5	21600	0.103	57992462.5	21600	0.474

Table 4.2: Results of the ANOVA analyses comparing respiration rates, photosynthetic rates and surface area between species and habitats

	Respiration rates			Photosynthetic rates			Surface area		
Factor	F	df	*p*	F	df	*p*	F	df	*p*
Species	1540.475	1	<0.0005	33.311	1	<0.0005	3375.77	1	<0.0005
Habitat	5.656	1	0.023	4.04	1	0.052	0.352	1	0.556

4.4.2 3D photogrammetry

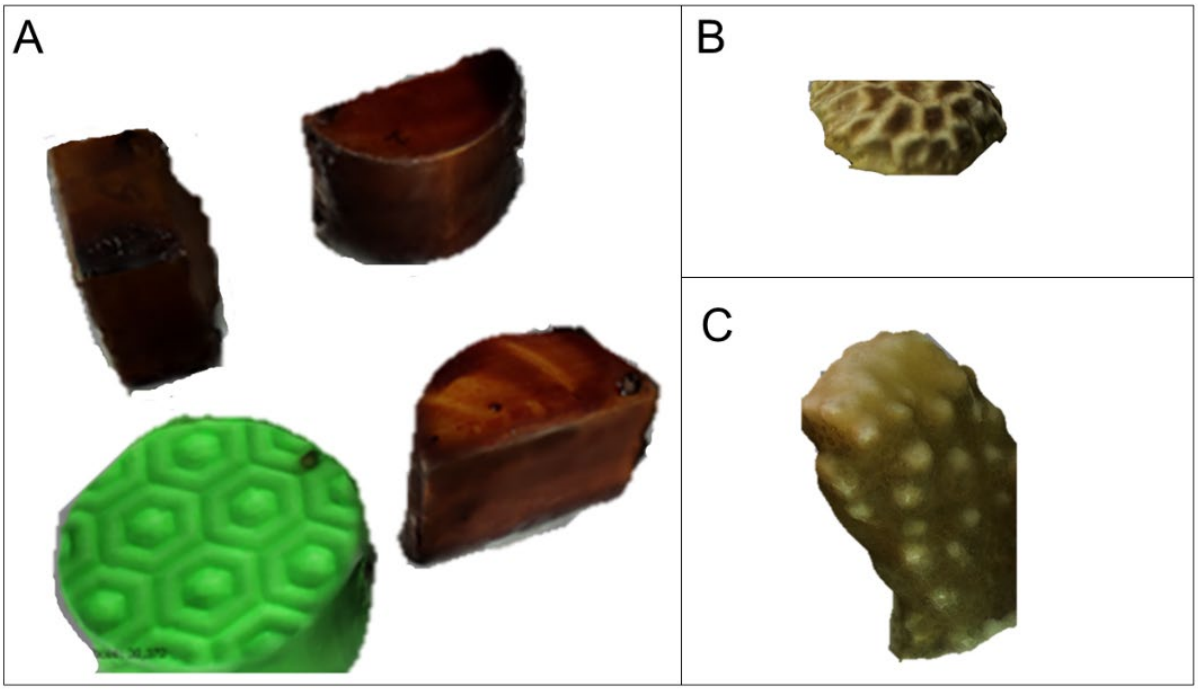

Figure 4.4: 3D outputs from Agisoft Metashape **A** objects of known surface area, **B** an *Anomastraea irregularis* fragment, **C** a *Pocillopora verrucosa* fragment

The 3D models constructed using Agisoft Metashape were successful for all fragments and objects with good detail of the surfaces (**Figure 4.4**). There were no significant differences in the surface area measurements of wooden and plastic objects attained from physical measurements and from Agisoft Metashape (**Figure 4.5a**, **Table 4.3**). There was a significant difference in the surface area between the two species (**Figure 4.5b, Table 4.2, Appendix 18**). The surface area of the fragments did not significantly differ between habitats (**Figure 4.5b, Table 4.2, Appendix 18**). The average surface area ± 1 standard deviation obtained using the 3D software were 31.83 ± 0.91 cm^2 for intertidal *A. irregularis*, 31.08 ± 0.56 cm^2 for subtidal *A. irregularis*, for 57.39 ± 1.96 cm^2 intertidal *P. verrucosa* and 57.60 ± 1.74 cm^2 for subtidal *P. verrucosa* (**Figure 4.5b**). The related-samples Friedman's test (Friedman's test statistic=0.255, df=2, p=0.880) indicated that the surface area of the wooden objects was not significantly different from the three methods. The paired-samples t-tests for both species (AI: t=-7.834, df=9, p<0.0005; PV: t=-20.475, df=9, p<0.0005) indicated that the wax dip method yielded significantly higher surface area measurements than the 3D photogrammetry method.

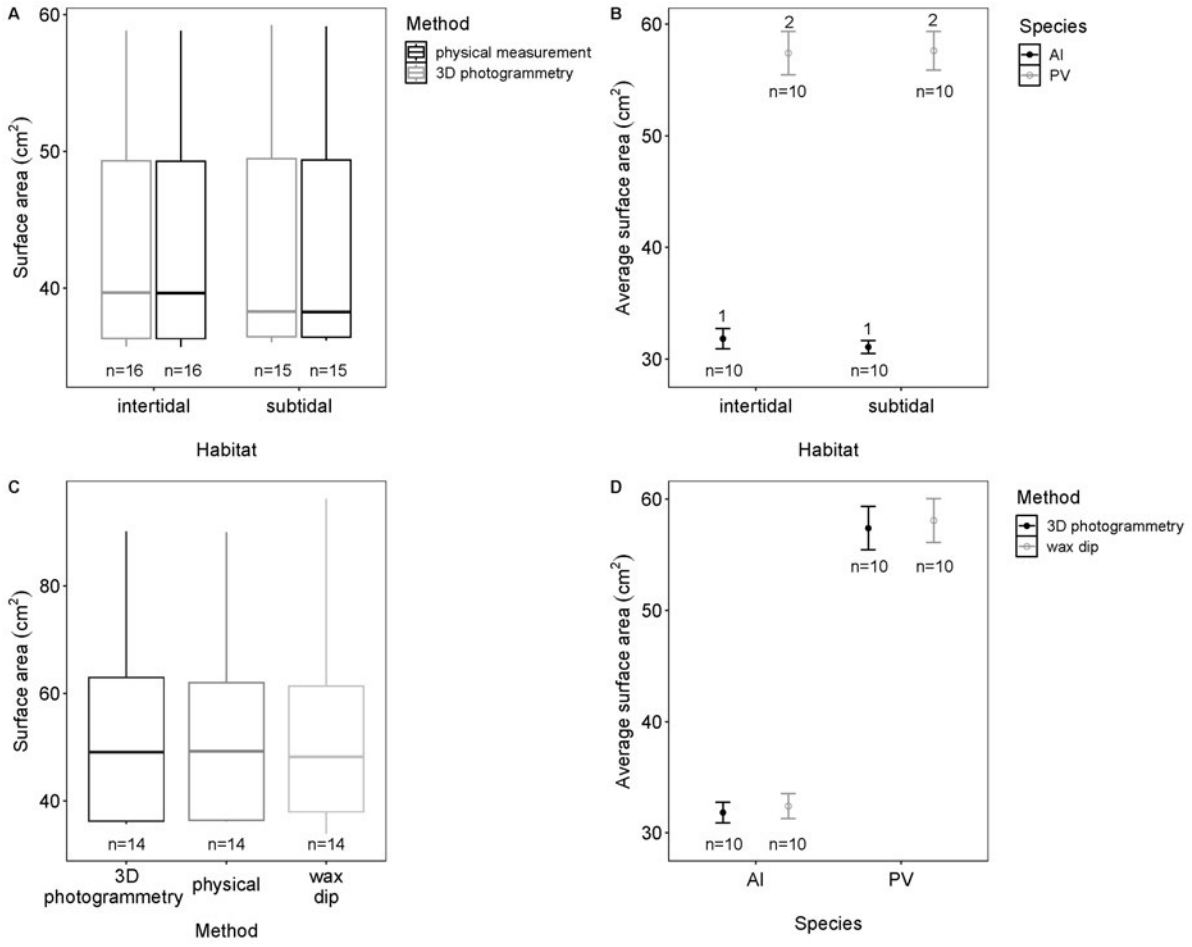

Figure 4.5: A Surface area (cm^2) measurements of wooden and plastic objects using the two different methods **B** average (±1SD) surface area (cm^2) of coral fragments from the two habitats attained from Agisoft Metashape, numbers (1 and 2) indicate the Bonferroni pairwise differences (**Appendix 18**) **C** surface area measurements of the rectangular wooden blocks attained from three methods **D** average surface (±1SD) of coral fragments attained from the 3D photogrammetry and wax dip methods

Table 4.3: Wilcoxon Signed Ranks test comparing between physical and 3D measurements of surface area (cm^2) of objects stuck in the intertidal and subtidal habitats

	Intertidal	Subtidal
Z-statistic	-1.762	-1.591
n	16	15
p	0.078	0.112

4.5 Discussion

The adapted flexi-chamber design was confirmed to be watertight and did not cause significant changes in the environmental seawater conditions during the 3-hour incubation (**Figure 4.2, Table 4.1**). The incubation of the coral fragments was also minimally invasive as no noticeable stress signs were witnessed during and after the incubation. The respiration and photosynthetic rates of small individual coral fragments can be measured directly *in situ* from fluxes of dissolved oxygen concentrations using the adapted flexi-chamber method. To the authors' knowledge, this is the first-time respiration and photosynthesis of *Pocillopora verrucosa* were measured *in situ* in this region, while reports of *Anomastraea irregularis* respiration/photosynthetic rates were not found anywhere in literature. Although variation in environmental conditions makes comparisons difficult, *P. verrucosa* respiration/photosynthetic rates measured during this study were within ranges of *P. verrucosa* fragments measured in other locations using complex traditional rigid chambers (Beisiegel 2012; Kegler et al. 2015).

There was a significant difference in respiration and photosynthesis between species (**Figure 4.3, Table 4.2**), highlighting that this method can capture variability in the responses of species *in situ*. Coral respiration and photosynthetic rates may vary due to several factors, such as coral morphology (tissue and skeleton thickness), the number of algal cells, chlorophyll-a content per algal cell or reliance on heterotrophy/autotrophy (Osinga et al. 2012). *A*nomastraea *irregularis* may rely more on heterotrophy than photosynthesis to meet its metabolic needs (Smit 2014; Boodraj and Glassom 2022), which may explain the lower photosynthetic rates

but higher respiration rates during the dark incubation. Subtidal *A. irregularis* had higher respiration rates compared to intertidal conspecifics (**Figure 4.3, Appendix 18**), which may have been due to the different environmental conditions experienced in the two different habitats (**Figure 4.2**). Temperature, light and water movement have been found to influence coral respiration and photosynthetic rates (Anthony and Hoegh-Guldberg 2003; Hennige et al. 2010; Faxneld et al. 2011; Camp et al. 2015). Subtidal corals of both species in this study also had noticeably lower photosynthetic rates than the intertidal conspecifics, but there was large variation around the means and therefore not statistically significantly different (**Appendix 18**). A larger sample size may have reduced the uncertainty in the habitat effect on photosynthetic rates. The variation in metabolic rates between species and habitats warrants further investigation to better explain the variable responses and understand how the different rates effects their performances.

Measurements of the surface area of the wooden and plastic objects were not significantly different using Vernier callipers as opposed to using the 3D software (**Figure 4.5a, Table 4.3**). This showed that the 3D software can give accurate results of surface area of objects of differing morphology and texture. The surface area of the coral fragments was successfully attained using the 3D photogrammetry method. There was a significant difference in the surface area between species (**Figure 4.5b, Appendix 18**). The method also seemed more precise for *A. irregularis* than *P. verrucosa* based on the ± 1 standard deviation obtained. This is understandable since *P. verrucosa* has a more complex morphology than the massive species *A. irregularis* (Figueira et al. 2015; Lange and Perry 2020; Million et al. 2021).

The surface area measurements of the rectangular wooden blocks did not significantly differ from the three methods of physical measurement using Vernier callipers, wax dipping, or 3D photogrammetry. The wax dip method produced significantly higher surface area measurements than the 3D photogrammetry method for both species. Conley and Hollander (2021) found similar results with the wax dip producing overestimations in surface area of coral fragments but not giving significantly different surface area measurements of wooden objects measured using 3D photogrammetry. It is believed that the 3D photogrammetry method gives more accurate surface area measurements for coral fragments and that the overestimation of the surface area measurements from the wax dip method is a result of the coral skeletons holding wax differently enough to cause discrepancies (Conley and Hollander 2021).

There are some considerations when using these two methods. It is important to note that PAR is what the Symbiodiniaceae use for photosynthesis. The transmission of PAR is important to take into consideration because the photosynthetic rates measured would be impacted if the material of the flexi-chamber used reduces the PAR significantly (Camp et al. 2015). The flexi-chamber used in this study did not significantly reduce PAR (**Figure 4.2**). However, it is noteworthy that if a material that affects PAR is used, PAR would be similarly affected for all treatments/habitats, any effects would change the photosynthesis reading, but not whether there was a difference between treatments/habitats. A flexible bicycle tyre inner tube filled with sand and fishing weights/stones (Mallon et al. 2022) can be a simpler alternative to secure the bag around the coral fragments *in situ* without needing to attach a concrete disk. This method of creating a seal is particularly useful in marine protected areas where modifications to the substrate is restricted (Mallon et al. 2022). Quantitative leakage tests described by Camp et al. (2015) and Mallon et al. (2022) may be preferable in future studies. It is also important to time the tidal cycles well when working with the flexi-chamber in the intertidal habitat so that the appropriate volume of seawater surrounding the fragments can be maintained during the incubation period.

With the advancement of 3D photogrammetry software any inexpensive camera with an underwater housing can be used to shoot coral fragments if it can focus on the coral fragments. Agisoft Metashape is not a free software however a 30-day free trial is available. Lange and Perry (2020) lists the benefits of using Agisoft Metashape and provides a video tutorial on how to use the software to measure coral growth. However, Meshroom and Meshlab are free software alternatives that can be used together to attain 3D models. Approximately 5 minutes were spent manually aligning images for a few point clouds when automatic alignment did not result. Automated targets from the software can be printed and attached to the reference rulers to speed up the alignment process, as the targets will be detected automatically by the software (Lange and Perry 2020). More precise alignments would result in more accurate surface area measurements that would aid in measuring growth rates.

Ultimately this study showed that the adapted flexi-chamber and the 3D photogrammetry method can be used to measure metabolic rates and growth of coral fragments of different morphology and from different habitats. No special complex equipment or training was required to attain the physiological results in this study. The methods were non-invasive and did not require destroying the coral fragments. Only two people were needed to conduct this study. The overall cost of this study was under ~ZAR20 000. This is important during this

period of great coral loss, where we need to efficiently study various coral species from different locations to gain more knowledge of their resilience/susceptibility to stressors.

4.6 Conclusion

Studying fundamental physiological processes of coral fragments *in situ* aids in reef management. This study showed that the flexi-chamber and 3D photogrammetry methods can be effectively used to measure the respiration, photosynthesis, and growth of small individual coral fragments of different morphology and habitat. The combination of the two methods can allow for more knowledge of fundamental physiological processes to be attained at lower financial cost and complexity.

Chapter 5: The effect of reciprocal transplantation between an environmentally variable and moderate habitat on the physiology of corals *Anomastraea irregularis* and *Pocillopora verrucosa*

5.1 Abstract

Increased sea surface temperatures due to global climate change are rapidly decimating global coral cover. Understanding the physiological responses of corals when exposed to thermal stress in their natural environments, especially extreme habitats, yields information that can be used to better conserve them. An *in-situ* experiment was conducted during the austral spring and summer months to ascertain the tolerance/susceptibility of corals from two environmentally varying habitats. *Anomastraea irregularis* (massive morphology) and *Pocillopora verrucosa* (branching morphology) were reciprocally transplanted between the environmentally variable intertidal and more stable subtidal habitats on the east coast of South Africa. Controls were established by placing fragments back in their original habitats. Respiration, photosynthesis, growth rates and coral health scores were measured monthly for six months. The Symbiodiniaceae density, chlorophyll-a concentration, chlorophyll-a concentration per symbiont cell, and lipid concentration were also analysed at the start and end of the study. There were differential physiological responses between species and habitat in response to the reciprocal transplant. Intertidal *Anomastraea irregularis* and *Pocillopora verrucosa* transplanted into the subtidal habitat showed potential acclimation to the subtidal habitat. These fragments were able to adjust their P:R ratios and maintain higher Symbiodiniaceae cell density, Symbiodiniaceae chlorophyll-a concentration, chlorophyll-a concentration per cell, and lipid content, experiencing less bleaching and mortalities. However, these fragments also experienced lower growth rates than the control fragments. *Anomastraea irregularis* appeared more physiologically plastic (altering respiration rates and maintaining higher Symbiodiniaceae cell density and lipid concentration) and, therefore, tolerant (less bleaching and mortalities) than *Pocillopora verrucosa* to the changes in environmental conditions. The results are promising since these tolerant corals may be used in conservation initiatives. The results of this study show the ability of some coral species to acclimatise and/or adapt to different environmental conditions, however, the potential for these corals to acclimatise/adapt to global climate change-related stressors still warrants further investigation especially since several other stressors are also impacting reef systems.

Keywords: respiration, photosynthesis, bleaching, lipid, Symbiodiniaceae

5.2 Introduction

The symbiosis between Symbiodiniaceae and reef-building corals is responsible for the productivity of reef ecosystems (Brown 1997; Stat et al. 2012). The coral-Symbiodiniaceae symbiosis is, therefore, vital for the functioning of reefs but is sensitive and can break down when the corals experience biological or environmental stress (Baker et al. 2008; Wooldridge 2017). Coral bleaching is the resulting phenomenon and is increasingly occurring due to increased sea surface temperatures due to anthropogenically induced climate change (Hughes et al. 2003; Pandolfi et al. 2011; Hughes et al. 2017b; Eddy et al. 2021). When tropical reefs experience a temperature of 1-2°C or more above the mean summer maximum for four weeks or more, significant bleaching events usually occur (Lesser 1997; Eakin et al. 2009; Richards et al. 2015).

Global mass bleaching events are occurring increasingly frequently due to increased thermal stress associated with global climate change (Hoegh-Guldberg et al. 2018; Ainsworth et al. 2021; Baum et al. 2023). Global mass bleaching events means that significant coral bleaching has been confirmed in all ocean regions (Atlantic, Pacific and Indian Ocean basins) where warm-water corals exist within a year (NOAA Coral Reef Watch). Coral cover started declining with thermal events since the 1970s however records of global coral cover was sparse prior to the 1990s (Souter et al. 2021). It is estimated that from 1997 to 2018 global coral cover has already decreased from 36 to 19%, with the Western Atlantic and Central Pacific experiencing the most severe losses (Tebbett et al. 2023). The 1998 global mass bleaching event saw a 8% decline in global coral cover and subsequent events occurring between 2009 and 2018 saw a decline of 14% (Souter et al. 2021). NOAA (2024) confirmed the 4th global coral bleaching event which started in the Northern Hemisphere summer of 2023 and followed in the Southern Hemisphere summer of 2023-2024. This mass bleaching event marks the second event in the last decade (Reimer et al. 2024). There is now consensus within the scientific community that corals natural ability to adapt to climate change is too slow and that reef ecosystems will experience significant decline in biodiversity, productivity, and complexity with the loss of corals (Alvarez-Filip et al. 2013; van Oppen et al. 2017; Hughes et al. 2018b; Baums et al. 2019; Vargas-Ángel et al. 2019; Eddy et al. 2021; Ford et al. 2023). In addition to the ecological devastation coral reef stakeholders such as coastal communities, fishers, and the tourism industry will also be severely impacted (Hoegh-Guldberg et al. 2018; McLeod et al. 2021). Research efforts have thus increased to find ways to speedily restore and conserve reefs to lessen the damage that will occur with the changing climate.

The large variability in thermal tolerance of corals is key to the persistence of coral reefs however the underlying physiological mechanisms that drive the variable tolerance is still not well understood and necessitates further investigation, especially in understudied species of differing morphology that persist in locations that are on the fringe of optimum environmental limits for scleractinian corals. Furthermore, building a comprehensive catalogue of coral performances is one of the first steps towards identifying resilient corals that can then be used in coral conservation projects (McClanahan et al. 2007; van Oppen et al. 2017; Baums et al. 2019; Quigley et al. 2020). Therefore, essential physiological processes namely respiration, photosynthesis, and growth needs to be assessed in various coral taxa from diverse locations to give indications of the coral's performance under varying environmental conditions and its future bleaching responses (Yap et al. 1998; Rinkevich 2005; Sawall et al. 2011; McClanahan et al. 2014; Baums et al. 2019; Camp et al. 2019; Morikawa and Palumbi 2019).

Laboratory thermal stress experiments may underestimate the physiological responses of corals to climate change because of the static nature of the thermal manipulations (Mayfield et al. 2012). In contrast, *in situ* reciprocal transplant experiments between habitats are advantageous as they allow for complex responses to be attained since the environmental variables are not static and naturally fluctuate (Oliver and Palumbi 2011; Mayfield et al. 2012; Safaie et al. 2018). These types of studies have highlighted that temperature variability can influence the thermal niche of corals. Habitats with high temperature variability were found to enhance the thermal resilience of some coral species through physiological adaptations and phenotypic variation (Oliver and Palumbi 2011; Palumbi et al. 2014; Safaie et al. 2018; Schoepf et al. 2020). For example, through reciprocal transplant experiments between a high-stress habitat and a lower-stress offshore habitat, *Porites lobata* were found to have physiological and molecular adaptations to the high-stress nearshore habitat (Tisthammer et al. 2021). The nearshore corals did not experience changes in their proteome or physiology between sites, while the offshore corals responded to nearshore transplantation with increased concentrations of proteins associated with detoxification, antioxidant defence, and regulation of cellular metabolic processes and decreased tissue layer thickness and growth rates (Tisthammer et al. 2021). Reciprocally transplanted corals between two reef habitats with more and less variable temperature regimes also revealed distinct transcriptomic differences that influence the higher bleaching thresholds and survival of thermally tolerant corals from highly variable environments (Palumbi et al. 2014; Bay and Palumbi 2017). Many corals from extreme habitats were found to maintain their thermal tolerance when transplanted to moderate habitats for

several months while corals from moderate habitats did not cope well with transplantation to extreme habitats (Palumbi et al. 2014; Morikawa and Palumbi 2019; Schoepf et al. 2019). Corals from thermally stable habitats may be adapted to a small thermal range and thus lack adaptations and the acclimatisation potential to increased thermal stress. The acclimatisation of tolerant corals to new environments has the potential to provide substantial thermal resistance to vulnerable coral populations quicker than natural selection would produce (Palumbi et al. 2014).

However, the ability and extent to which tolerant coral species from extreme habitats can acclimate or adapt to new conditions remain unclear (Palumbi et al. 2014). Species-specific mechanisms and regional thermal anomalies can influence the thermal tolerance of corals from extreme habitats, and therefore, these transplanted corals may not provide immunity to extreme thermal stress events (Schoepf et al. 2015b; Schoepf et al. 2019; Klepac and Barshis 2020). There is evidence that some tolerant corals may experience trade-offs in growth when transplanted to new environments (Howells et al. 2013; Bay and Palumbi 2017) while some do not experience any trade-offs (Barott et al. 2021). Therefore, the ability of thermally tolerant corals to acclimate or adapt to new environmental conditions without significant growth or fitness costs is an important factor in evaluating their potential to serve as sources of thermal resilience for vulnerable coral populations. The aim of this study was to better understand the thermal tolerance/susceptibility of a branching species, *Pocillopora verrucosa*, and a massive species, *Anomastraea irregularis*, from the environmentally variable intertidal pools and environmentally stable subtidal habitat at Treasure Beach, South Africa. It was hypothesised that corals from the dynamic intertidal habitat would have greater physiological plasticity and resilience to transplantation stress compared to those from the stable subtidal habitat. This was achieved by reciprocally transplanting fragments of both species between the two habitats and measuring selected physiological rates and tissue composition over six months over austral spring and summer. Understanding the physiological response of these high-latitude corals, especially from the extreme intertidal habitats, can be useful for future conservation efforts.

5.3 Materials and methods

5.3.1 Collection of corals, experimental design, and transplant of corals

The thick-tissue massive species *Anomastraea irregularis* and the thin-tissue branching species *Pocillopora verrucosa* were selected for this study as they are both common on the Southeastern coast of KwaZulu-Natal (Smit and Glassom 2017). The experiment was performed from 25 September 2022 to 12 March 2022 (**Appendix 19**), which coincides with

the region's warmer months. The austral spring and summer are known for higher temperatures and increased solar radiation, factors directly linked to thermal stress in corals, making it the most stressful time of year for corals (McClanahan et al. 2005; Brown and Dunne 2016; Heron et al. 2016). Conducting the study during warmer months allowed for capturing natural thermal tolerance/susceptibility of the corals when stressed by transplantation to new thermal regimes. Two *A. irregularis* (3.08 ± 0.18 cm) and two *P. verrucosa* (3.10 ± 0.11 cm) fragments were taken randomly from ten healthy colonies in the highly variable (intertidal rock pools ~0.39 m depth) and ten healthy colonies in the more stable (subtidal ~3 m depth) habitats (**Appendix 19** for experimental tree diagram) at Treasure Beach (29°57'32.8"S 30°59'19.0"E) in KwaZulu-Natal on the East Coast of South Africa. A hammer and a chisel were used to collect the *A. irregularis* fragments, while wire cutters were used to the collect the *P. verrucosa* fragments (under the South African Department of Environmental Affairs and the Department of Agriculture, Forestry and Fisheries research permit number RES2023-54). According to the University of KwaZulu-Natal's Animal Research Ethics Committee Standard Operating Procedures no other permission or ethical approval was required for this study.

Each fragment was stuck onto a concrete disk as described in chapter 4. Ten fragments of each species were attached back into their habitat of origin to act as controls and are therefore referred to as native II (intertidal to intertidal) or native SS (subtidal to subtidal) (**Appendix 19**). Ten fragments of each species from the intertidal habitat were transplanted into the subtidal habitat and are referred to as foreign IS (intertidal to subtidal) (**Appendix 19**). Ten fragments of each species from the subtidal habitat were transplanted into the intertidal habitat and are referred to as foreign SI (subtidal to intertidal) (**Appendix 19**). Two weeks were given for the fragments to recover from handling before physiological measurements were performed. Five blank concrete disks were attached in each habitat as described in chapter 4. A temperature data logger (iButtons Maxim®, United States of America) was placed in each habitat and were set to record temperatures at 30-minute intervals (Smit and Glassom 2017). The loggers were coated with a thin layer of opaque silicone for waterproofing and attached with epoxy putty (Pratley® Quickset Putty, South Africa) in natural crevices away from direct sunlight and were never exposed to the air at spring low tide (Bahr et al. 2016; Smit and Glassom 2017).

5.3.2 Determination of coral health and physiological rates

The health of the coral fragments was monitored visually using the Coral Health Chart (Coral Watch, Australia) (Siebeck et al. 2006) during low tide at six sampling occasions: 08 October 2022, 24 November 2022, 19 December 2022, 25 January 2023, 20 February 2023, and 12

March 2023. The photosynthetic and respiration rates were determined on the same six sampling occasions using the modified flexi-chamber method, as described in Chapter 4. The surface area of each fragment was obtained using the three-dimensional (3D) photogrammetry method as described in Chapter 4. The growth rates of the fragments were determined as described by Lange and Perry (2020).

5.3.3 Measurement of tissue composition

At the start (08 October 2022) and end (12 March 2023) of the experiment, three fragments of each species from each habitat from the control and treatment groups were removed. The fragments were flash-frozen in liquid nitrogen and transported on ice to the laboratory at the University of KwaZulu-Natal. The coral tissue from each fragment was then removed and processed as described in Chapter 2 to measure the Symbiodiniaceae cell density, chlorophyll-a content, chlorophyll-a per Symbiodiniaceae cell, and lipid content.

5.3.4 Statistical analysis

For each species, five Generalised Estimating Equations (GEE) were run to assess whether there were significant differences in coral health score, respiration, photosynthesis, P:R, and growth rates between habitats (intertidal and subtidal) and origin (control and foreign) at the different times (six sampling occasions). GEEs were performed using gamma distribution with log link functions as the residuals were nonparametric (one sample Kolmogorov-Smirnov on residuals of the dependent variables $p < 0.05$). The colony effect was added as a covariate to account for random effects but was removed from the models if it was insignificant.

For each species, four ANOVAs were performed to assess whether there were significant differences in Symbiodiniaceae cell density, Symbiodiniaceae chlorophyll-a concentration, chlorophyll-a per Symbiodiniaceae cell, and lipid concentration between habitats (intertidal and subtidal) and origin (control and foreign) at the two times (start and end of experiment). The residuals were parametric and had homogenous variance (one sample Kolmogorov-Smirnov on residuals of the dependent variable $p > 0.05$ and ANOVA on residuals of the dependent variable $p = 1.000$). Bonferroni pairwise comparisons using estimated marginal means were employed to determine statistical differences within tested groups. No statistical analyses were performed on the temperature data since there was only one data logger placed in each habitat. However, the daily temperature variability trends and degree heating weeks (Liu et al. 2006) were calculated for each habitat over time and plotted in R (R Development Core Team, http://www.R-project.org/).

5.4 Results

5.4.1Temperature trends

The mean daily temperature and ranges generally increased through the summer months and was more pronounced in the intertidal habitat than the subtidal habitat (**Figure 5.1a**). Overall, the intertidal habitat had 1.26 times higher mean daily temperature, and 1.42 times greater variability compared to the subtidal habitat (**Figure 5.1a**). The highest maximum daily range in the intertidal habitat was recorded in March 2023 (mean 5.67 ± 0.72 standard error) and the lowest was recorded in October 2022 (mean 1.50 ± 0.21 standard error) (**Figure 5.1b**). Contrastingly the highest maximum daily range in the subtidal habitat was recorded in January 2023 (mean 3.06 ± 0.31 standard error) and the lowest was recorded in March 2023 (mean 1.45 ± 0.35 standard error) (**Figure 5.1b**). Overall, the intertidal habitat had 1.42 times higher maximum daily temperature range than the subtidal habitat (**Figure 5.1b**). Although the degree heating weeks for both habitats were below 4 throughout the study period, there was a general increase from October 2022 to March 2023. This increase was more pronounced in the intertidal habitat compared to the subtidal habitat (**Figure 5.1c**). Overall, the intertidal habitat had a mean degree heating week approximately 4.24 times higher than the subtidal habitat.

5.4.2 Bleaching and mortality

Native fragments of both species in both habitats remained healthy, with no mortality recorded throughout the study (**Figure 5.2**). A total of six coral fragments were missing/lost during the experiment (**Appendix 20**). After one month, an SI *Pocillopora verrucosa* fragment died, but there were no other mortalities at that time (**Appendix 20**). After two months, one *P. verrucosa* SI fragment and one *A. irregularis* SI fragment died (**Appendix 20**). No IS fragments died during the experiment (**Appendix 20**). From the 24th of November 2022 (a month into the experiment) until the end of the experiment (12 March 2023), the coral scores of the SI fragments of both species (*A. irregularis* SI: mean 5.27 ± 0.03 standard error, *P. verrucosa* SI: mean 4.78 ± 0.15 standard deviation) were significantly lower than the native fragments (*A. irregularis* II: mean 6.00 ± 0.00 standard error and SS: mean 5.91 ± 0.04 standard error, *P. verrucosa* II: mean 5.95 ± 0.03 standard error and SS: mean 6.00 ± 0.00 standard error) (**Figure 5.2a, Table 5.1, Appendix 21**). Conversely, during the same period, the coral scores of IS fragments of both species (*A. irregularis* IS: mean 5.89 ± 0.05 standard error, *P. verrucosa* IS: mean 5.81 ± 0.06 standard deviation) did not significantly differ from the native fragments but were significantly higher than the SI fragments (**Figure 5.2a, Table 5.1, Appendix 21**). For both species, the reaction norms further highlighted that overall, there was a significant

interaction effect of origin and habitat on the coral health score (**Figure 5.2b, Table 5.1, Appendix 21**). Overall, for both species, IS fragments maintained similar coral scores to the native fragments (II and SS), whereas SI fragments exhibited significantly lower coral scores compared to the natives and IS. Specifically, for *A. irregularis* SI coral score was ~12% lower than II, ~11% lower than SS, and ~12% lower than IS (**Figure 5.2b, Table 5.1, Appendix 21**). For *P. verrucosa* SI coral score was ~20% lower than II, 20% lower than SS, and 22% lower than IS (**Figure 5.2b, Table 5.1, Appendix 21**). Overall, the coral scores of *P. verrucosa* IS fragments were approximately 1% lower than *A. irregularis* IS fragments, and *P. verrucosa* SI fragments were approximately 9% lower than *A. irregularis* SI fragments (**Figure 5.2b**).

5.4.3 Physiological responses

Over the duration of this study the respiration rates, photosynthetic rates, and P:R ratios did not significantly differ for both species (**Table 5.1, Figure 5.3**). The GEE for *A. irregularis* respiration rate revealed a significant interaction effect of habitat and origin (**Table 5.1**) which can be seen in the reaction norm (**Figure 5.4a**). The Bonferroni pairwise test (**Appendix 22**) further showed that this was because the SI *A. irregularis* fragments (mean 0.01883 ± 0.00006 standard error) had higher respiration rates than the native fragments (II: mean 0.01862 ± 0.00005 standard error, SS: mean 0.01845 ± 0.00004 standard error), while the IS fragments (mean 0.01846 ± 0.00007 standard error) had similar respiration rates to the SS fragments (mean 0.01845 ± 0.00004 standard error) (**Figure 5.4a**). II *A. irregularis* fragments were found to have ~0.9% significantly higher respiration rates when compared to the SS fragments (**Figure 5.4a, Appendix 22**). Conversely, the respiration rates of *P. verrucosa* fragments did not significantly differ between habitats nor between origin during the study (**Figure 5.3a, Table 5.1**). *Anomastraea irregularis* native fragments from both habitats had ~74% higher respiration rates than the *P. verrucosa* fragments (**Figure 5.4a**).

The GEE for *A. irregularis* and *P. verrucosa* gross photosynthetic rates showed a significant habitat and origin interaction effect (**Table 5.1, Figure 5.4b**). The Bonferroni pairwise test (**Appendix 22**) further showed that this was because the SI fragments of both species (*A. irregularis*: mean 0.00710 ± 0.00008 standard error, *P. verrucosa*: mean 0.01507 ± 0.00004) had significantly lower gross photosynthetic rates than the native fragments (*A. irregularis* II: mean 0.00745 ± 0.00006 standard error and SS: mean 0.00738 ± 0.00006 standard error, *P. verrucosa* II: mean 0.01530 ± 0.00008 standard error and SS: mean 0.01520 ± 0.00003 standard error) as well as the IS fragments (*A. irregularis*: mean 0.00719 ± 0.00006 standard error, *P. verrucosa*: mean 0.01522 ± 0.00003). *Pocillopora verrucosa* IS fragments had similar gross

photosynthetic rates to the SS fragments, while *A. irregularis* IS fragments had ~2.57% significantly lower gross photosynthetic rates than their SS fragments (**Figure 5.4b**). The P:R (gross photosynthetic rate: respiration rate) ratios of both species also followed the same pattern, with the SI fragments (*A. irregularis*: mean 0.37771 ± 0.00548 standard error, *P. verrucosa*: mean 1.40805 ± 0.00511) having significantly lower ratios than the native fragments (*A. irregularis* II: mean 0.40071 ± 0.00442 standard error and SS: mean 0.40023 ± 0.00389 standard error, *P. verrucosa* II: mean 1.43464 ± 0.00940 standard error and SS: mean 1.42318 ± 0.00342 standard error) as well as the IS fragments (*A. irregularis*: mean 0.39017 ± 0.00445 standard error, *P. verrucosa*: mean 1.42433 ± 0.00469) (**Figure 5.4c**, **Table 5.1**, **Appendix 22**). For both species, IS fragments had similar P:R ratios to the SS fragments (**Figure 5.4c**, **Table 5.1**, **Appendix 22**). *Pocillopora verrucosa* fragments appeared to have ~109% higher average gross photosynthesis and resultant P:R ratios than *A. irregularis* (**Figure 5.4c**).

The GEE for *A. irregularis* and *P. verrucosa* growth rates showed a significant interaction effect of time and habitat (**Table 5.2**). Transplantation significantly impacted the growth rates of *A. irregularis* and *P. verrucosa*, and at certain times there was a significant difference in the growth rates of the native and foreign fragments in both habitats (**Figure 5.5a, Table 5.2, Appendix 23**). The Bonferroni pairwise tests in **Appendix 23** show where the precise significant differences occurred. Generally, for both species, initial (October-November) growth rates did not significantly differ between habitats, and the native fragments had higher growth rates than the foreign fragments (*A. irregularis*: II mean 0.0274 ± 0.0076 standard error, SS mean 0.0334 ± 0.0043 standard error, SI mean 0.0030 ± 0.0118 standard error, IS mean 0.0122 ± 0.0063 standard error; *P. verrucosa*: II mean 0.0274 ± 0.0039 standard error, SS mean 0.0182 ± 0.0072 standard error, SI mean 0.0071 ± 0.0131 standard error, IS mean 0.0091 ± 0.0078 standard error).However, after December, growth rates significantly increased, with the highest rates in February (*A. irregularis*: II mean 0.0769 ± 0.0000 standard error, SS mean 0.0962 ± 0.0131 standard error, SI mean 0.0833 ± 0.0064 standard error, IS mean 0.0549 ± 0.0165 standard error; *P. verrucosa*: II mean 0.1026 ± 0.0081 standard error, SS mean 0.1026 ± 0.0128 standard error, except for SI mean 0.0000 ± 0.0322 standard error, IS mean 0.0641 ± 0.0162 standard error) and then dropped in March(*A. irregularis*: II mean 0.0600 ± 0.0100 standard error, SS mean 0.0500 ± 0.0000 standard error, SI mean 0.0333 ± 0.0167 standard error, IS mean 0.0643 ± 0.0143 standard error; *P. verrucosa*: II mean 0.0500 ± 0.0316 standard error, SS mean 0.0917 ± 0.0083 standard error, SI mean 0.0400 ± 0.0400 standard error, IS

mean 0.0750 ± 0.0112 standard error) but remained above the initial growth rates (**Figure 5.5a, Appendix 23**). Overall, *A. irregularis* IS fragments (0.0389 ± 0.0094) had ~31% significantly lower growth rate than SS (0.0566 ± 0.0096) while SI (0.0423 ± 0.0132) had although not statistically significant ~16% lower growth rate to II (0.0503 ± 0.0088) (**Figure 5.4b and Appendix 23**). Conversely, overall, for *P. verrucosa* the growth rates were statistically similar for the natives (II: 0.0566 ± 0.0123 and SS: 0.0564 ± 0.0146) and foreign (IS: 0.0437 ± 0.0116 and SI: 0.0271 ± 0.0093) fragments in both habitats (**Figure 5.4b and Appendix 23**).

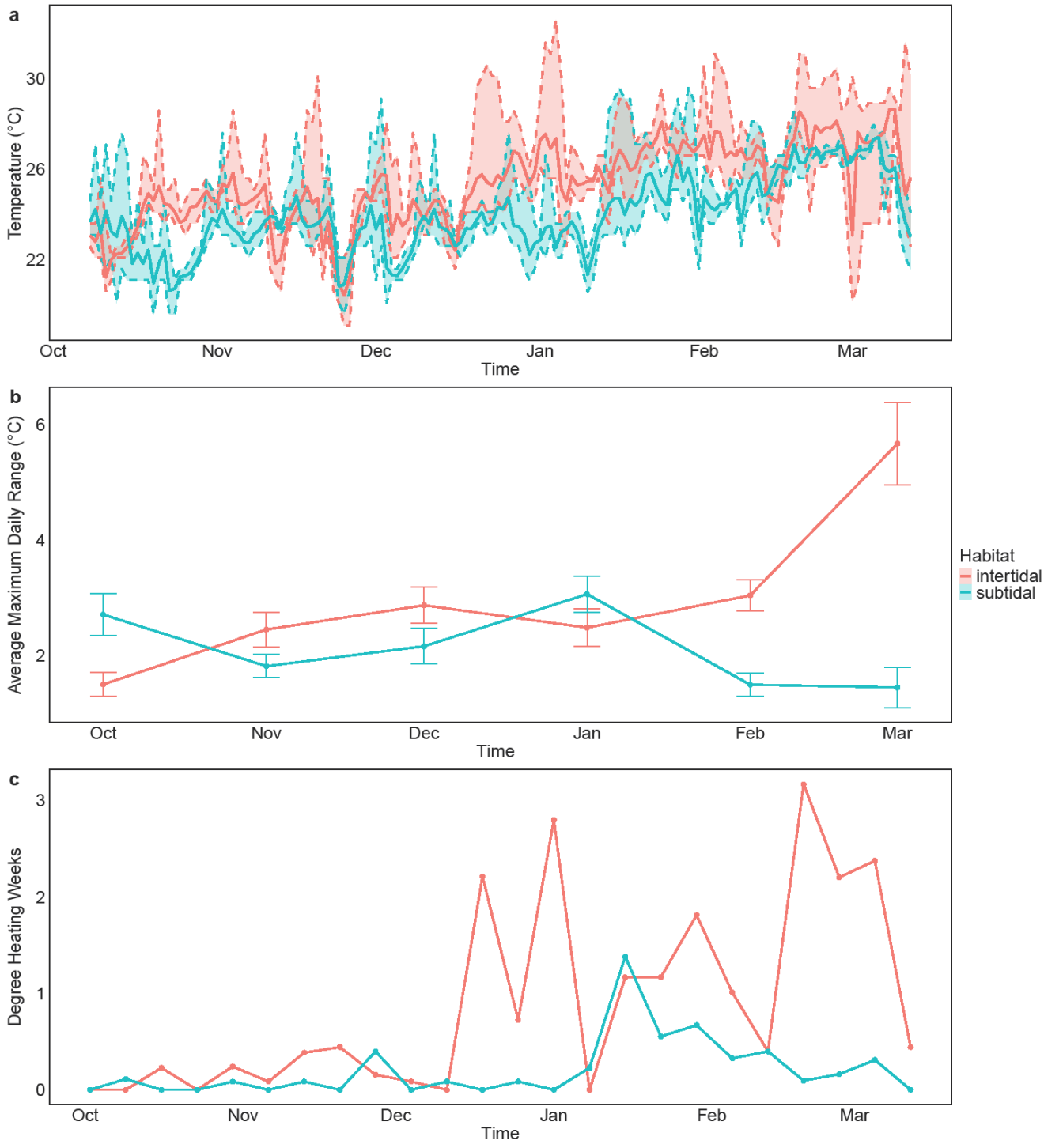

Figure 5.1: a The daily temperature variability for each habitat over time, mean (solid lines) with maximums and minimums (shaded areas), **b** The mean ± standard error maximum daily range for each habitat over the course of the study, **c** The degree heating weeks for each habitat over the course of the study

Table 5.1: GEE results for each species comparing the coral score and metabolic rates between habitat (intertidal and subtidal), origin (native and foreign), times (six sampling occasions). *indicates significant effects

		Anomastraea irregularis			*Pocillopora verrucosa*		
Physiological variable	Factor	Wald Chi-Square	df	*p*	Wald Chi-Square	df	*p*
Coral score	Time	50.28	5	<0.0005*	163.66	5	<0.0005*
	Habitat	10.45	1	<0.0005*	78.4	1	<0.0005*
	Origin	20.63	1	<0.0005*	118.46	1	<0.0005*
	Time x habitat	19.21	5	<0.0005*	103.17	5	<0.0005*
	Time x Origin	37.38	5	<0.0005*	125.69	5	<0.0005*
	Habitat x Origin	19.07	1	<0.0005*	65.39	1	<0.0005*
	Time x Origin x habitat	28.47	5	<0.0005*	71.94	5	<0.0005*
Respiration rate	Time	11.13	5	0.055	6.357	5	0.273
	Habitat	48.249	1	<0.0005*	0.08	1	0.777
	Origin	7.394	1	0.007*	0.861	1	0.354
	Time x habitat	2.9996	5	0.701	0.642	5	0.986
	Time x Origin	0.333	5	0.997	1.778	5	0.879
	Habitat x Origin	5.397	1	0.020*	0.71	1	0.399
	Time x Origin x habitat	0.941	5	0.967	1.04	5	0.959
Gross photosynthesis rate	Time	4.143	5	0.529	1.639	5	0.896
	Habitat	0.249	1	0.618	1.095	1	0.295
	Origin	32.576	1	<0.0005*	11.755	1	<0.0005*
	Time x habitat	1.367	5	0.928	1.808	5	0.875
	Time x Origin	0.902	5	0.97	3.005	5	0.699
	Habitat x Origin	4.315	1	0.038*	18.866	1	<0.0005*
	Time x Origin x habitat	6.069	5	0.3	5.471	5	0.361
P:R	Time	5.717	5	0.335	1.256	5	0.939
	Habitat	5.092	1	0.024*	0.801	1	0.371
	Origin	26.176	1	<0.0005*	10.015	1	0.002*
	Time x habitat	1.557	5	0.906	0.757	5	0.98
	Time x Origin	0.746	5	0.98	3.42	5	0.636
	Habitat x Origin	4.938	1	0.026*	14.452	1	<0.0005*
	Time x Origin x habitat	4.356	5	0.499	4.046	5	0.543

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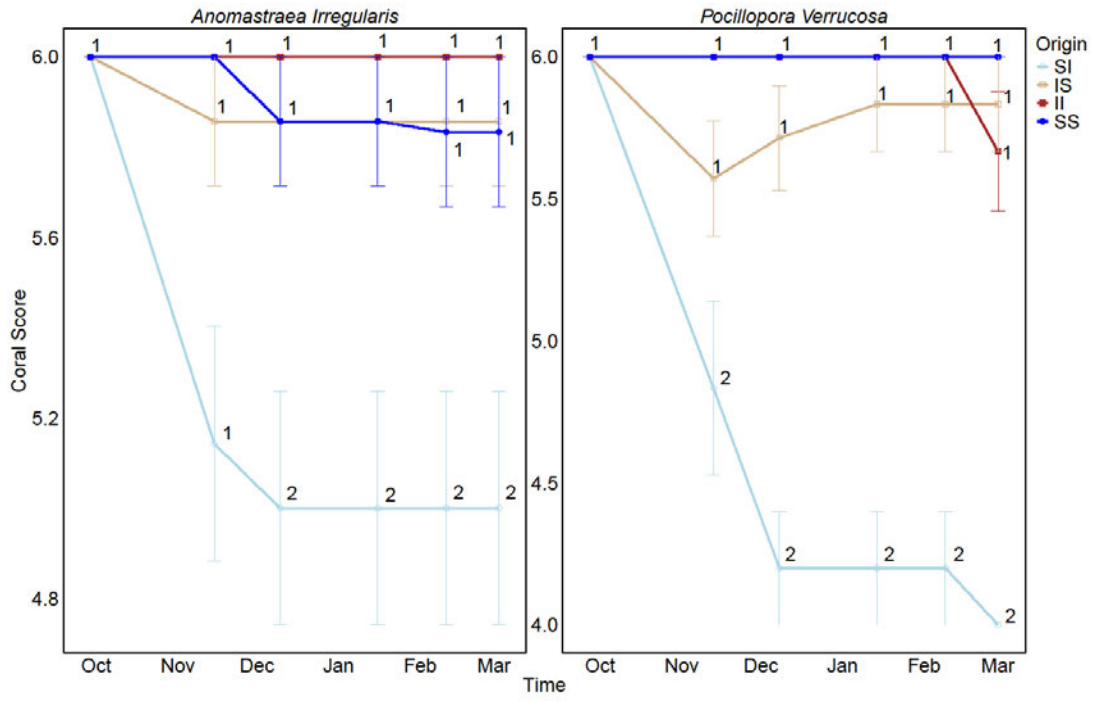

Anomastraea Irregularis
Pocillopora Verrucosa
Coral Score
6.0
5.6
5.2
4.8
5.5
5.0
4.5
4.0
Oct
Nov
Dec
Jan
Feb
Mar
Time
Origin
SI
IS
II
SS

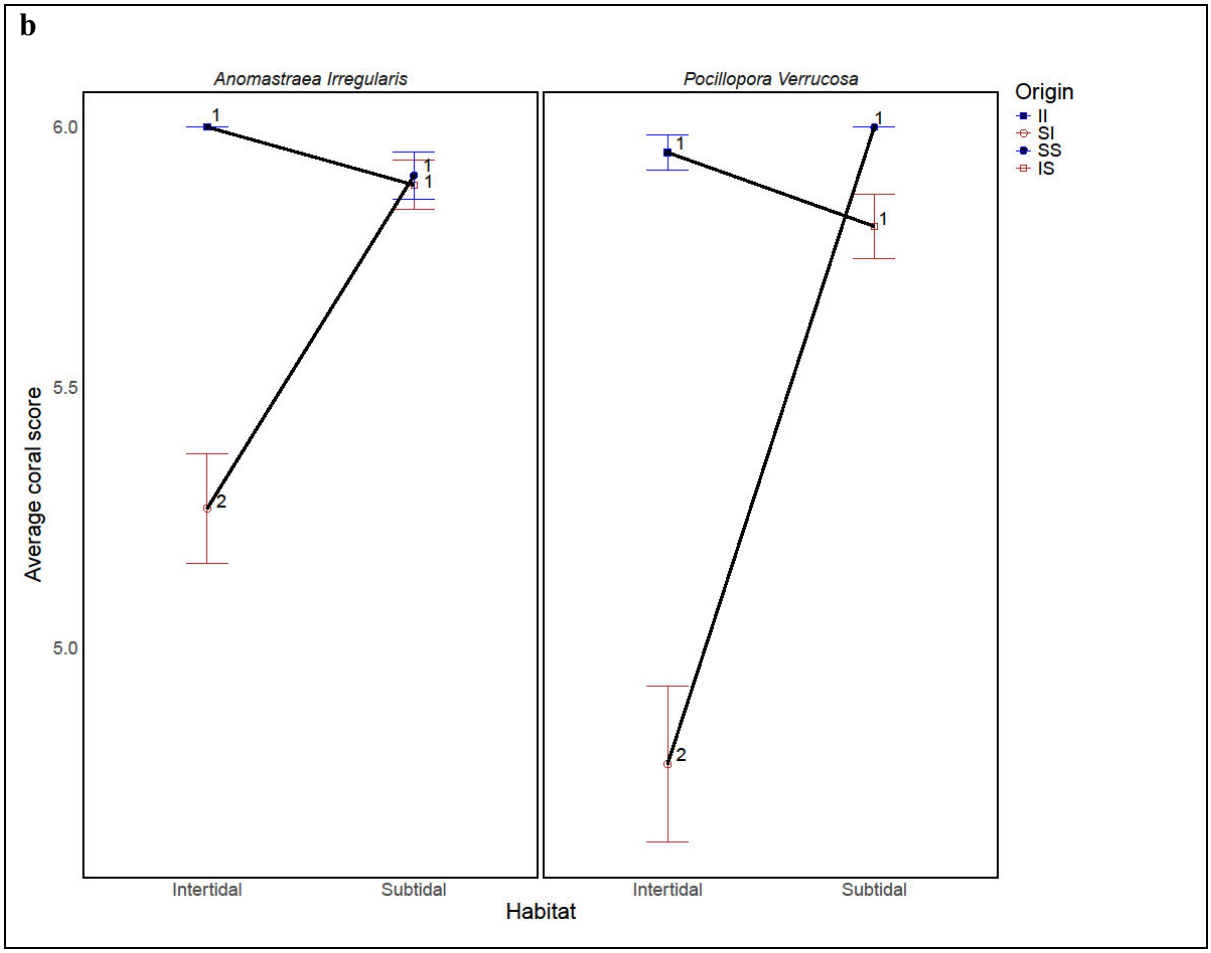


Figure 5.2: **a** Average coral health score ± 1 standard error for *Anomastraea irregularis* and *Pocillopora verrucosa* fragments transplanted in the intertidal and subtidal habitats over the course of the study, **b** Reaction norm showing the overall average coral score ± 1 standard error *Anomastraea irregularis* and *Pocillopora verrucosa* fragments across both habitats. Numbers (1 and 2) indicate significant differences amongst tested groups (II, SS, IS, and SI) for each species according to Bonferroni pairwise tests for the GEEs.

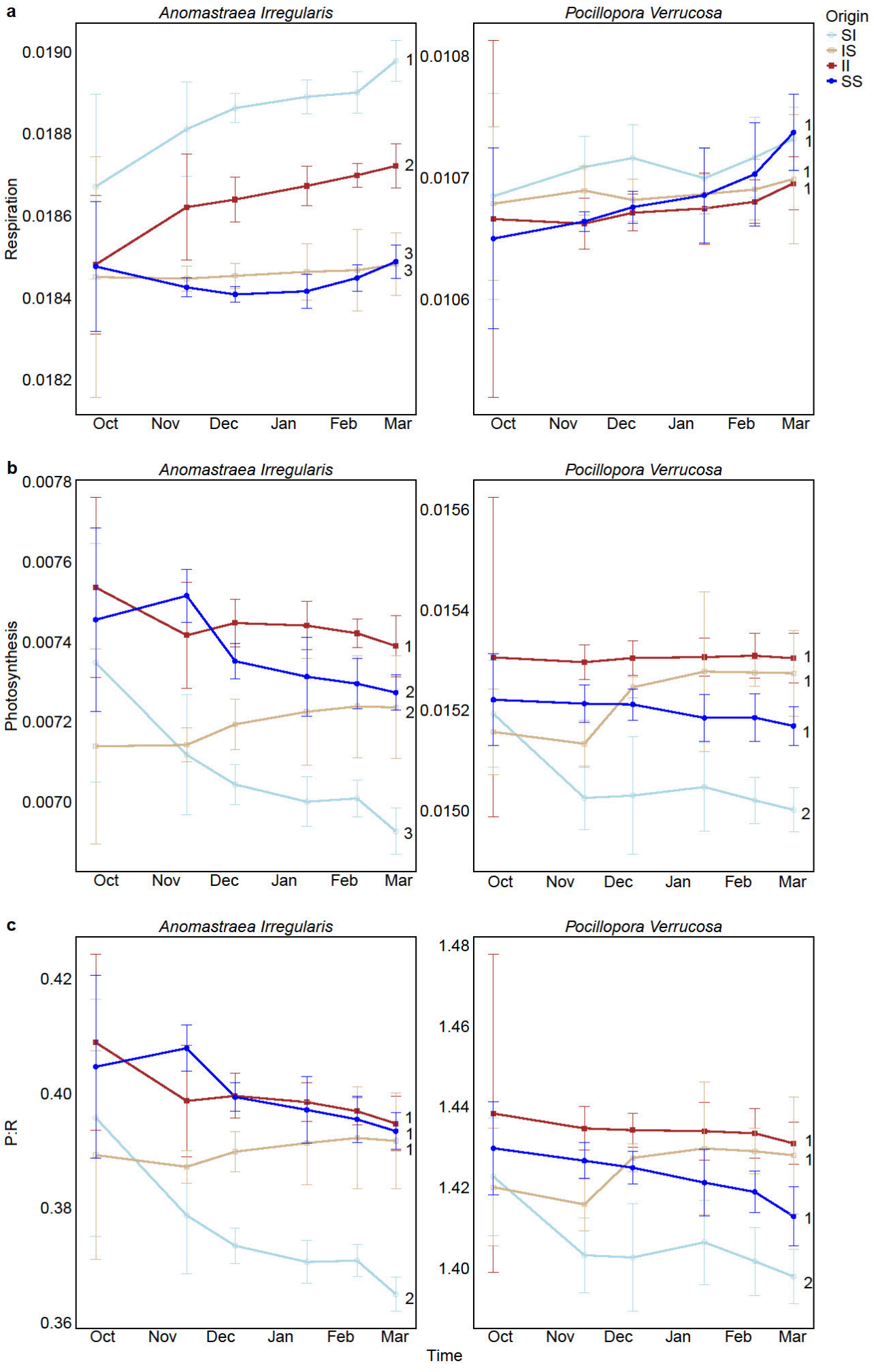
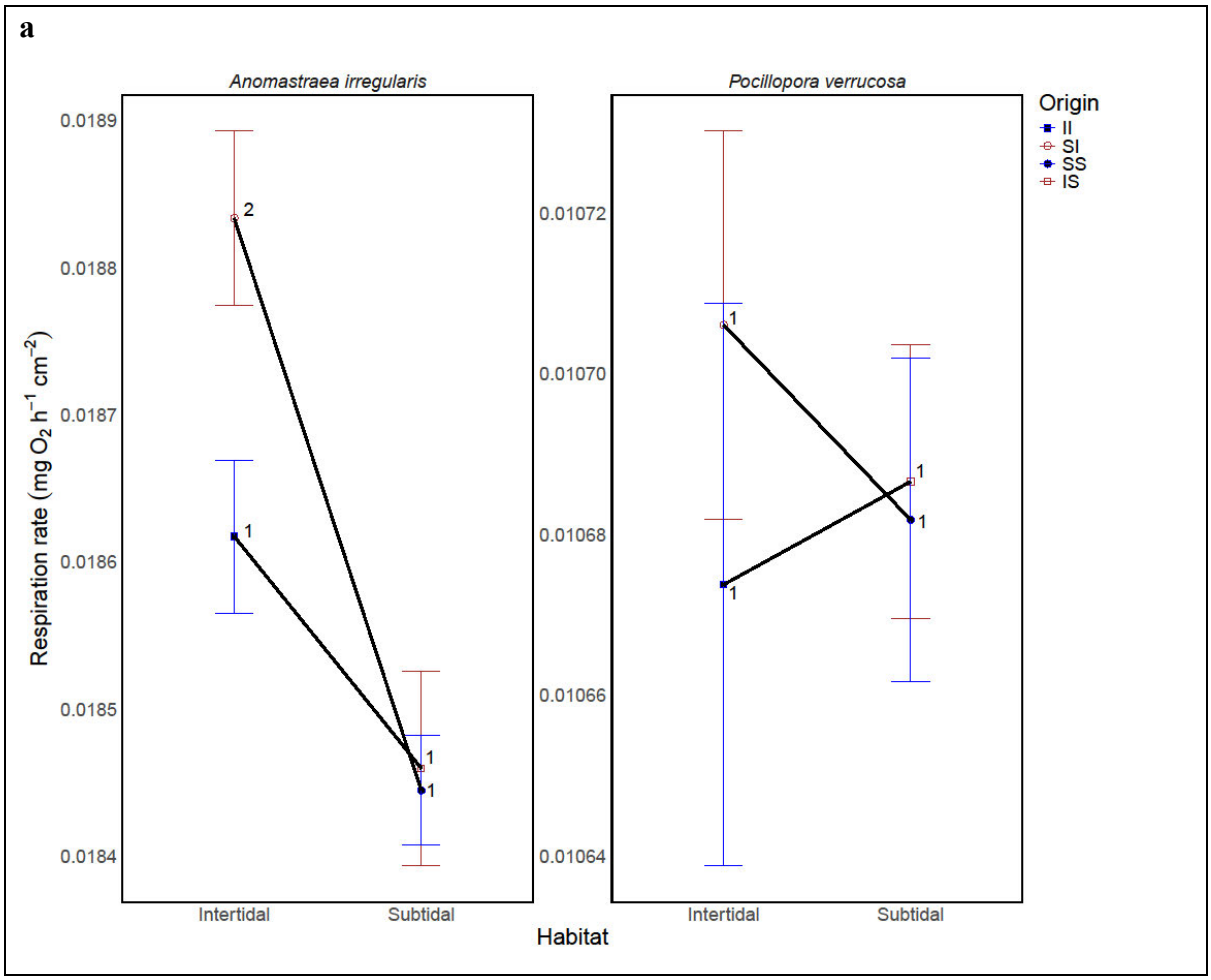
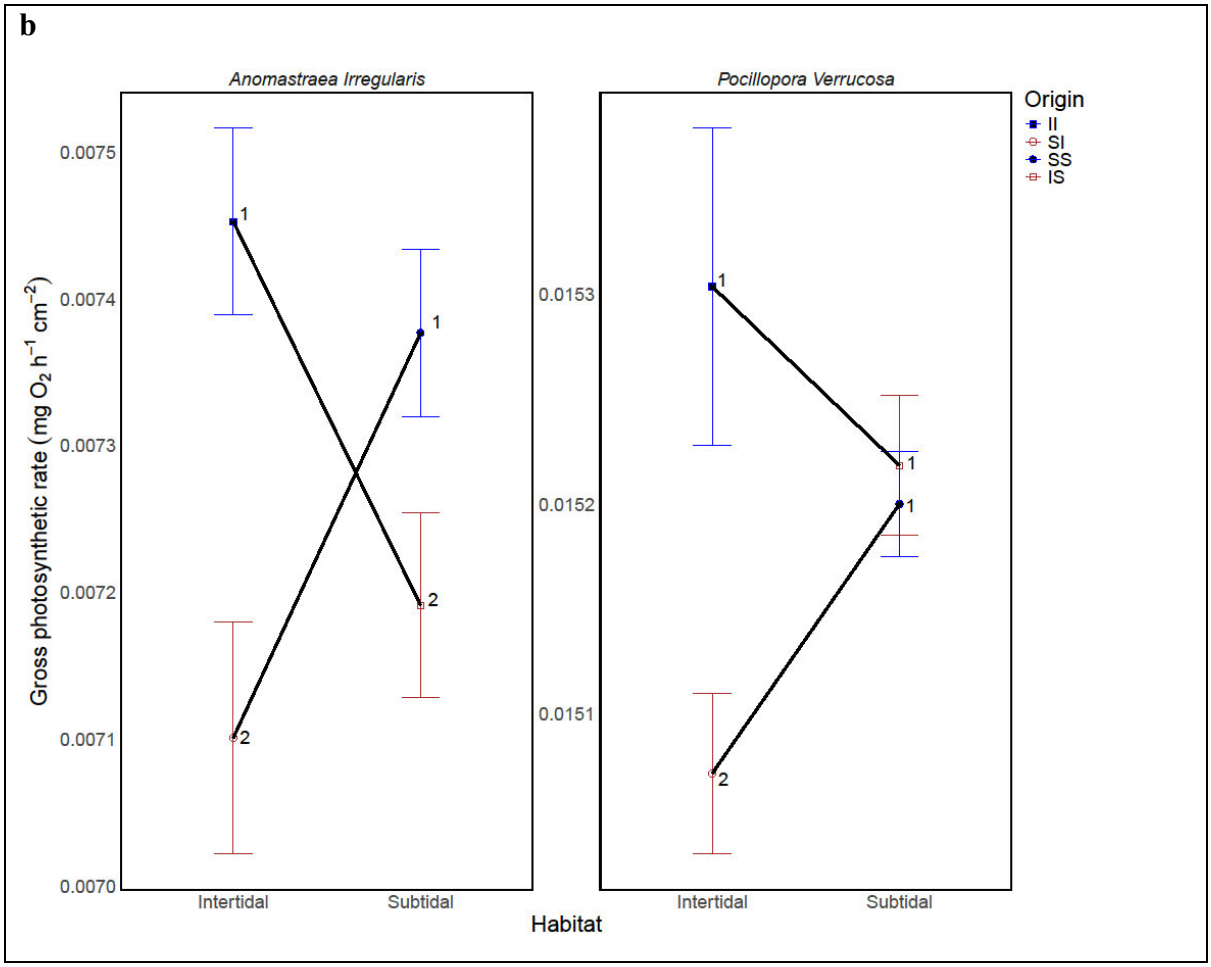

a
Anomastraea Irregularis
Pocillopora Verrucosa
Origin
SI
IS
II
SS
Respiration
0.0190
0.0188
0.0186
0.0184
0.0182
0.0108
0.0107
0.0106
Oct
Nov
Dec
Jan
Feb
Mar
b
Photosynthesis
0.0078
0.0076
0.0074
0.0072
0.0070
0.0156
0.0154
0.0152
0.0150
c
P:R
0.42
0.40
0.38
0.36
1.48
1.46
1.44
1.42
1.40
Time

Figure 5.3: **a** Average respiration (mg O_2 h^{-1} cm^{-2}) ± 1 standard error, **b** Average gross photosynthesis (mg O_2 h^{-1} cm^{-2}) ± 1 standard error, and **c** Average gross photosynthesis: respiration (P:R) ratio ± 1 standard error of *Anomastraea irregularis* and *Pocillopora verrucosa* fragments transplanted in the intertidal and subtidal habitats over the course of the study. Numbers (1, 2, and 3) indicate overall significant differences amongst tested groups (II, SS, IS, and SI) for each species according to Bonferroni pairwise tests for the GEEs.




b
Anomastraea Irregularis
Pocillopora Verrucosa
Origin
II
SI
SS
IS
Gross photosynthetic rate (mg O2 h-1 cm-2)
0.0075
0.0074
0.0073
0.0072
0.0071
0.0070
0.0153
0.0152
0.0151
Intertidal
Subtidal
Habitat

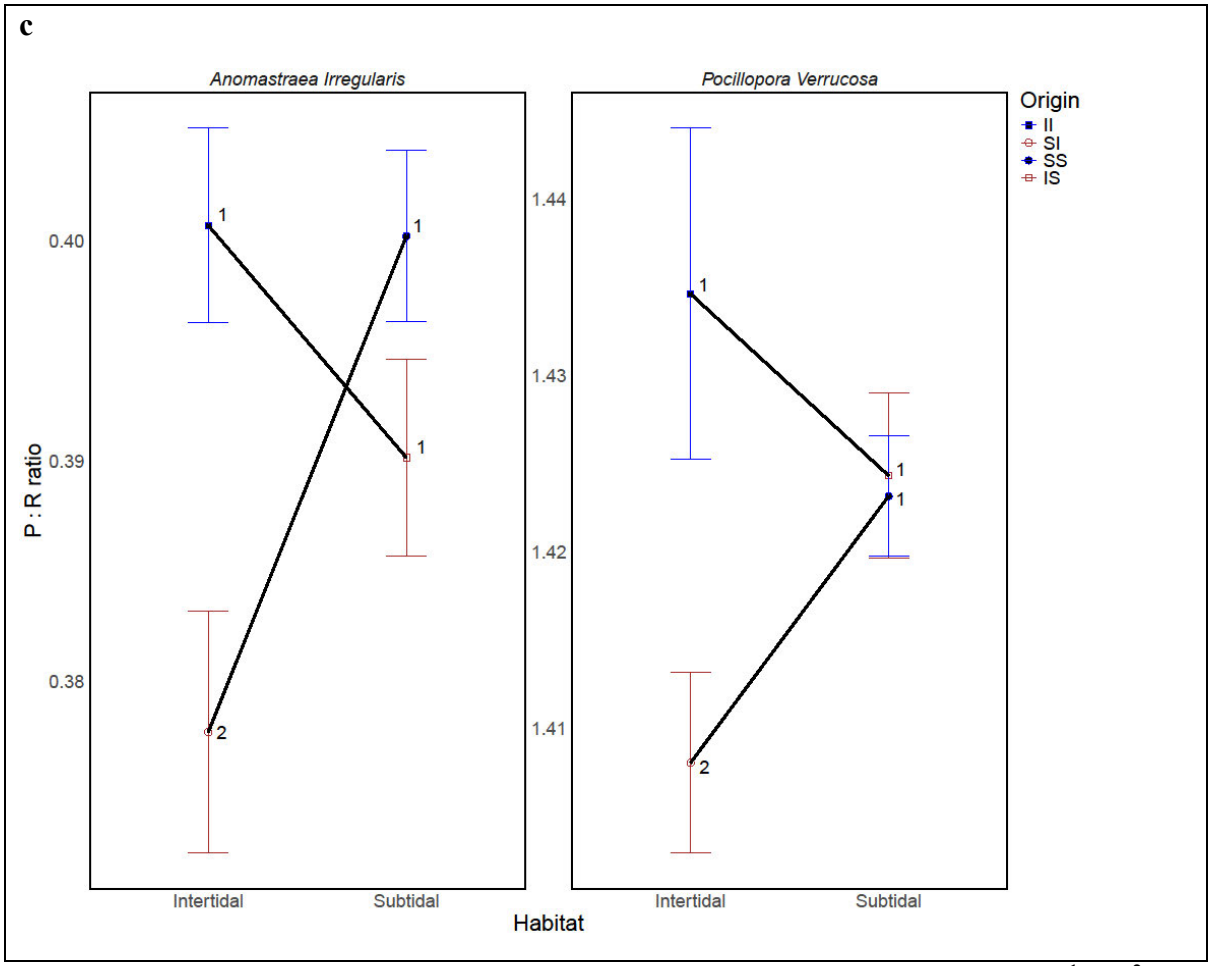


Figure 5.4: Reaction norms showing the overall **a** Average respiration (mg O_2 h^{-1} cm^{-2}) ± 1 standard error, **b** Average gross photosynthesis (mg O_2 h^{-1} cm^{-2}) ± 1 standard error, and **c** Average gross photosynthesis: respiration (P:R) ratio ± 1 standard error of *Anomastraea irregularis* and *Pocillopora verrucosa* fragments transplanted in the intertidal and subtidal habitats. Numbers (1 and 2) indicate significant differences amongst tested groups (II, SS, IS, and SI) for each species according to Bonferroni pairwise tests for the GEEs.

Table 5.2: GEE results for each species comparing the growth rate between habitat (intertidal and subtidal), origin (native and foreign), and times (five occasions). *indicates significant effects

	Anomastraea irregularis			*Pocillopora verrucosa*		
Factor	Wald Chi-Square	df	*p*	Wald Chi-Square	df	*p*
Time	136.385	4	<0.0005*	49.956	4	<0.0005*
Habitat	0.082	1	0.775	1.321	1	0.25
Origin	6.275	1	0.012*	8.73	1	0.003*
Time x habitat	9.767	4	0.045*	10.841	4	0.028*
Time x Origin	3.424	4	0.49	18.285	4	0.001*
Habitat x Origin	0.891	1	0.345	1.384	1	0.239
Time x Origin x habitat	8.297	4	0.081	4.072	4	0.396

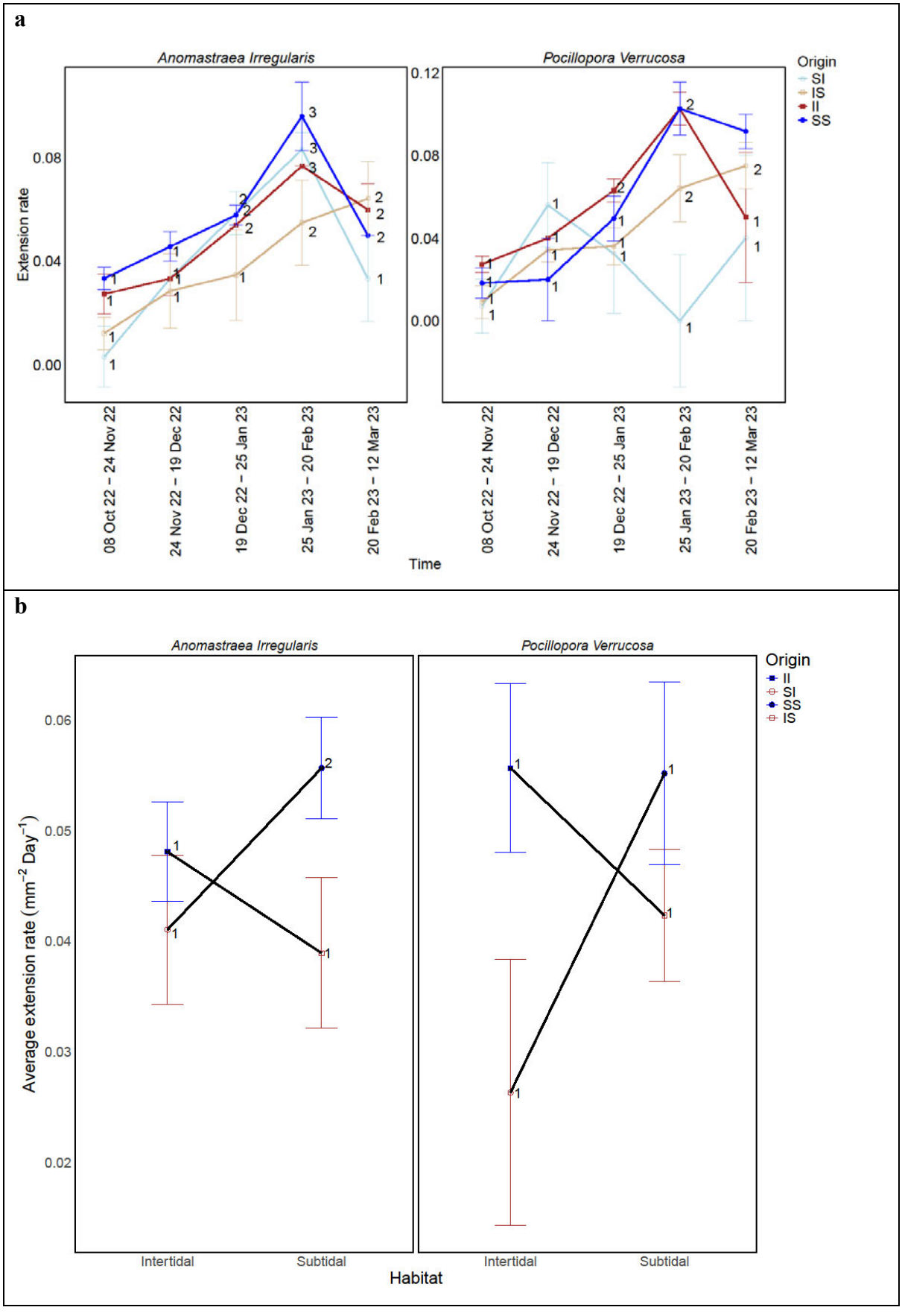

a
Anomastraea Irregularis
Pocillopora Verrucosa
Origin
SI
IS
II
SS
Extension rate
0.00
0.04
0.08
0.12
08 Oct 22 – 24 Nov 22
24 Nov 22 – 19 Dec 22
19 Dec 22 – 25 Jan 23
25 Jan 23 – 20 Feb 23
20 Feb 23 – 12 Mar 23
Time
b
Anomastraea Irregularis
Pocillopora Verrucosa
Origin
II
SI
SS
IS
Average extension rate (mm^{-2} Day^{-1})
0.02
0.03
0.04
0.05
0.06
Intertidal
Subtidal
Habitat

Figure 5.5: **a** Average growth rate (mm^{-2} day^{-1}) ± 1 standard error of *Anomastraea irregularis* and *Pocillopora verrucosa* fragments transplanted in the intertidal and subtidal habitats after ~ every month from the start until the end of the experiment, **b** Reaction norm showing the overall average growth rate (mm^{-2} day^{-1}) ± 1 standard error of *Anomastraea irregularis* and *Pocillopora verrucosa* fragments transplanted in the intertidal and subtidal habitats. Numbers (1, 2, and 3) indicate significant differences amongst tested groups (II, SS, IS, and SI) for each species according to Bonferroni pairwise tests for the GEEs.

5.4.4 Tissue composition

The four ANOVAs for both species' Symbiodiniaceae cell density, chlorophyll-a concentration, chlorophyll-a concentration per Symbiodiniaceae cell, and lipid concentration showed a significant interaction effect of habitat and origin (**Table 5.3**). The Bonferroni pairwise comparisons tests (**Appendix 24, 25, 26, and 27**) further showed that this was because overall the SI fragments of both species had significantly lower Symbiodiniaceae cell density (*A. irregularis*: ~7% and ~4% ; *P. verrucosa*: ~8% and ~7%), chlorophyll-a concentration (*A. irregularis*: ~27% and ~22%, *P. verrucosa*: ~23% and ~22), chlorophyll-a concentration per Symbiodiniaceae cell (*A. irregularis*: ~19% and ~18%, *P. verrucosa*: ~14% and ~14%), and lipid concentration (*A. irregularis*: ~26% and ~26%, *P. verrucosa*: ~11% and ~13%) than II fragments and IS fragments (**Figure 5.7, Appendix 24, 25, 26, and 27**). Furthermore, for both species, the Symbiodiniaceae cell density, chlorophyll-a concentration, chlorophyll-a concentration per Symbiodiniaceae cell and lipid concentration in IS fragments remained higher than the SS fragments (**Appendix 24, 25, 26, and 27, Figure 5.7**). The Symbiodiniaceae cell density, chlorophyll-a concentration, and chlorophyll-a concentration per Symbiodiniaceae cell in both species native fragments did not significantly differ between the start and end times (**Appendix 24**, **25, and 26, Figure 5.6a, b, and c**). *Anomastraea irregularis* IS fragments had ~5% significantly lower Symbiodiniaceae cell density and ~7% significantly lower chlorophyll-a concentration in at the end of the experiment as compared to the initial (**Figure 5.6a and b, Appendix 24 and 25**). Despite the reduction, overall, *A. irregularis* IS fragments still had ~3% significantly higher Symbiodiniaceae density, ~17% significantly higher chlorophyll-a content, and ~14% significantly higher chlorophyll-a per Symbiodiniaceae cell than their SS fragments (**Appendix 24**, **25, and 26, Figure 5.7a, b, and c**). However, the reduction meant that overall *A. irregularis* IS fragments had ~3% significantly lower Symbiodiniaceae density and ~4% significantly lower chlorophyll-a content compared to II (**Appendix 24 and 25, Figure 5.7a and b**). However, the chlorophyll-a concentration per

Symbiodiniaceae cell in the IS *A. irregularis* fragments did not significantly differ between the start and the end of the experiment (**Appendix 26, Figure 5.6c**), and remained similar to their II fragments overall (**Appendix 26, Figure 5.7c**).In opposition to *A. irregularis* IS fragments, no significant change was seen in the Symbiodiniaceae cell density, chlorophyll-a concentration, and chlorophyll-a concentration per Symbiodiniaceae cell in *A. irregularis* SI fragments between the two times and remained below the II values (**Appendix 24 and 25, Figure 5.6a and b**). The *P. verrucosa* SI fragments had ~2% significantly lower Symbiodiniaceae cell density and ~4% significantly lower chlorophyll-a concentration, and no change in chlorophyll-a concentration per Symbiodiniaceae cell at the end of the experiment than initial (**Appendix 24, 25, and 26, Figure 5.6a, b, and c**). No significant differences were seen in the same parameters in the P. *verrucosa* IS fragments between the two times (**Appendix 24, 25, and 26, Figure 5.6a, b, and c**). This meant that overall, the *P. verrucosa* IS fragments had ~6% significantly higher Symbiodiniaceae cell density, ~21% significantly higher chlorophyll-a concentration, and ~14% significantly higher chlorophyll-a concentration per Symbiodiniaceae cell than their SS fragments (**Appendix 24, 25, and 26, Figure 5.7a, b, and c**).

The lipid content in *A. irregularis* native fragments of both habitats did not significantly differ between the start and the end of the experiment (**Appendix 27**, **Figure 5.6d**). The lipid content of the *P. verrucosa* SS fragments similarly did not significantly differ between the two times. However, the lipid content in *P. verrucosa* II fragments was ~4% significantly lower at the end compared to the start of the experiment (**Appendix 27**, **Figure 5.6d**). At the end of the experiment, the lipid content in SI fragments of both species was significantly lower (*A. irregularis*: ~5% and *P. verrucosa*: ~6%) than their initial concentrations and therefore significantly lower than the SS fragments final concentrations (*A. irregularis*: ~4% and *P. verrucosa*: ~5%) (**Appendix 27**, **Figure 5.6d**). There was no significant difference in the lipid content of the IS fragments of both species at the end of the experiment when compared to the initial content (**Appendix 27, Figure 5.6d**). At the end of the experiment the lipid content in *P. verrucosa* IS fragments was ~3% significantly higher than the II fragments (**Appendix 27, Figure 5.6d**). There also seemed to be a difference in the tissue composition between species and habitat (**Figure 5.6**). Overall, in both species, the II fragments had higher tissue composition values than SS fragments (*A. irregularis*: Symbiodiniaceae cell density ~6%, chlorophyll-a content ~27%, chlorophyll-a per cell ~19%, lipid content ~23%; *P. verrucosa*: Symbiodiniaceae cell density ~6%, chlorophyll-a content ~21%, chlorophyll-a per cell ~14%,

lipid content ~8%) (**Figure 5.7, Appendix 24, 25, 26, and 27**). Overall, the Symbiodiniaceae cell density was ~27% and lipid concentration was ~64% higher in the *A. irregularis* fragments, while the chlorophyll-a concentration was ~26% and chlorophyll-a concentration per Symbiodiniaceae cell was ~42% higher in the *P. verrucosa* fragments (**Figure 5.7**).

Table 5.3: ANOVA results for each species comparing the Symbiodiniaceae cell density, Symbiodiniaceae chlorophyll-a concentration, and chlorophyll-a per Symbiodiniaceae cell ratio between habitat (intertidal and subtidal), origin (native and foreign), and times (at the start and end of the experiment). *indicates significant effects

		Anomastraea irregularis			*Pocillopora verrucosa*		
Variable	Factor	F statistic	df	*p*	F statistic	df	*p*
Symbiodiniaceae cell density	Time	5.769	1	<0.0005*	5.898	1	0.027*
	Habitat	3.2244	1	0.029*	2.469	1	0.136
	Origin	7.7	1	0.014*	4.193	1	0.057
	Time x habitat	3.224	1	0.091	2.469	1	0.136
	Time x Origin	3.746	1	0.071	1.2	1	0.29
	Habitat x Origin	66.993	1	<0.0005*	295.512	1	<0.0005*
	Time x Origin x habitat	2.589	1	0.127	1	1	0.332
Chlorophyll a concentration	Time	15.687	1	0.001*	15.439	1	0.001*
	Habitat	6.196	1	0.024*	1.385	1	0.256
	Origin	18.995	1	<0.0005*	2.646	1	0.123
	Time x habitat	6.496	1	0.021*	2.299	1	0.149
	Time x Origin	12.697	1	0.003*	4.309	1	0.054
	Habitat x Origin	1139.872	1	<0.0005*	2423.171	1	<0.0005*
	Time x Origin x habitat	3.991	1	0.063	1.018	1	0.328
Chlorophyll a concentration per Symbiodiniaceae cell	Time	0.808	1	0.382	3.39	1	0.084
	Habitat	0.073	1	0.79	0.016	1	0.899
	Origin	0.799	1	0.385	0.03	1	0.865
	Time x habitat	0.098	1	0.758	0.088	1	0.771
	Time x Origin	0.776	1	0.391	1.486	1	0.241
	Habitat x Origin	239.217	1	<0.0005*	1326.244	1	<0.0005*
	Time x Origin x habitat	0.006	1	0.938	0.1	1	0.756
Lipid content	Time	9.37	1	0.007*	37.76	1	<0.0005*
	Habitat	2.902	1	0.108	16.404	1	<0.0005*
	Origin	2.48	1	0.135	0.074	1	0.789
	Time x habitat	1.838	1	0.194	7.791	1	0.013*
	Time x Origin	2.854	1	0.111	1.866	1	0.191
	Habitat x Origin	1270.785	1	<0.0005*	329.682	1	<0.0005*
	Time x Origin x habitat	3.783	1	0.07	0.312	1	0.584

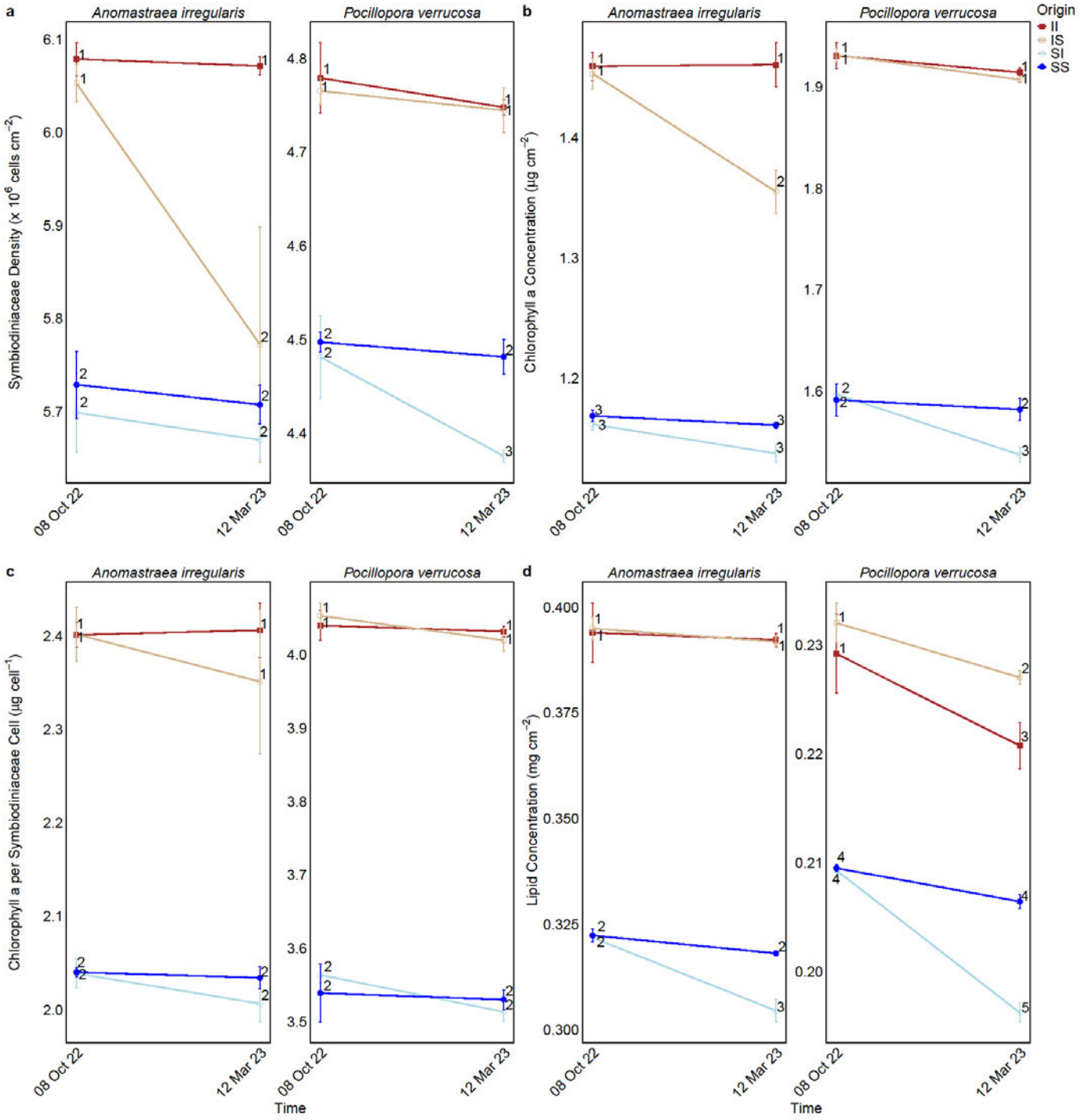
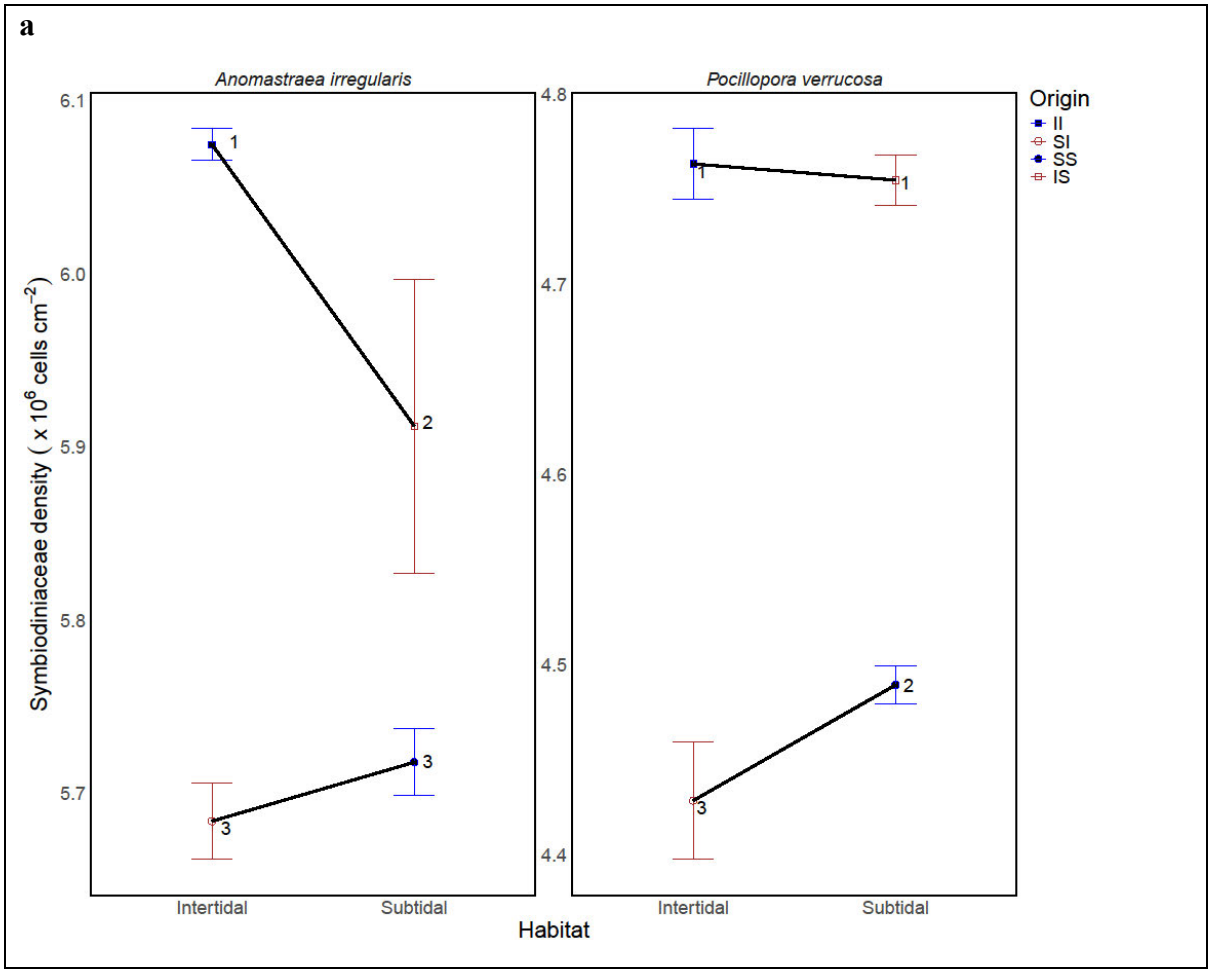
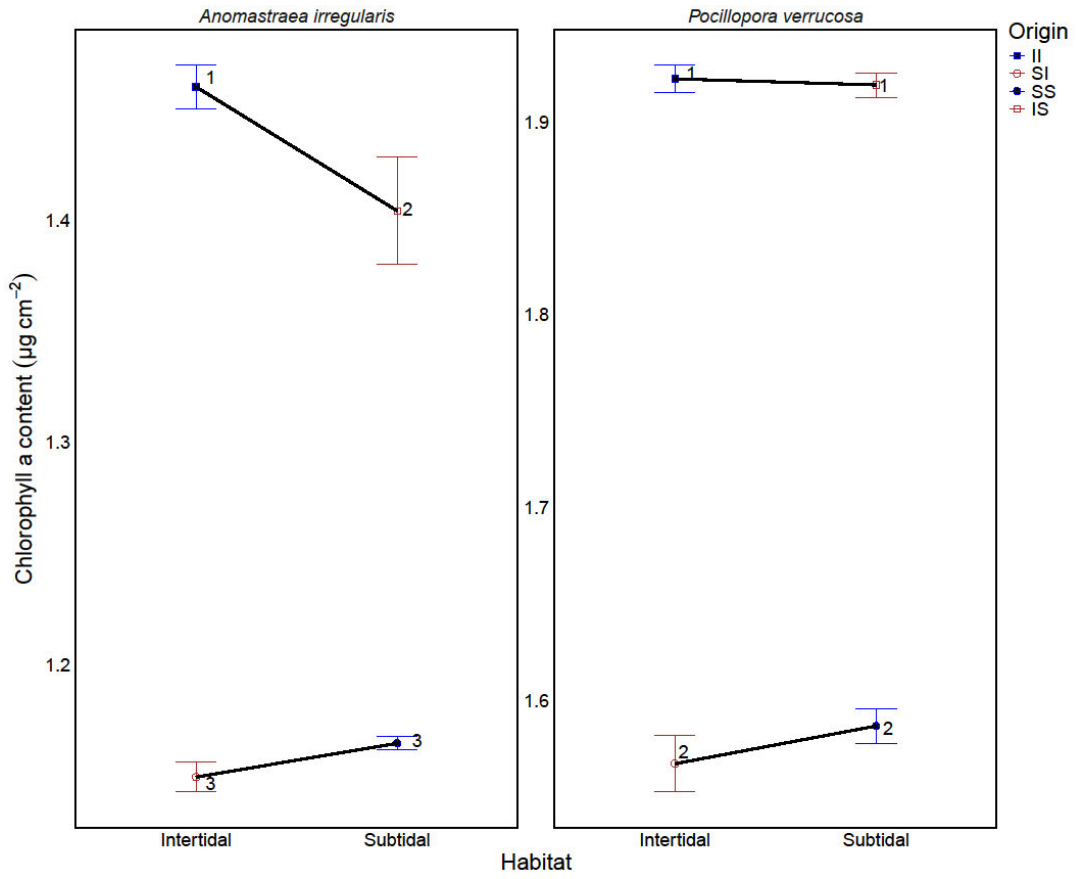


Figure 5.6: **a** Average Symbiodiniaceae cell density (number of cells. cm^{-2}) ± 1 standard error, **b** average chlorophyll-a concentration (µg. cm^{-2}) ± 1 standard error, **c** average chlorophyll per Symbiodiniaceae cell ± 1 standard error, and **d** average lipid concentration (mg. cm^{-2}) ± 1 standard error in *Anomastraea irregularis* and *Pocillopora verrucosa* fragments transplanted

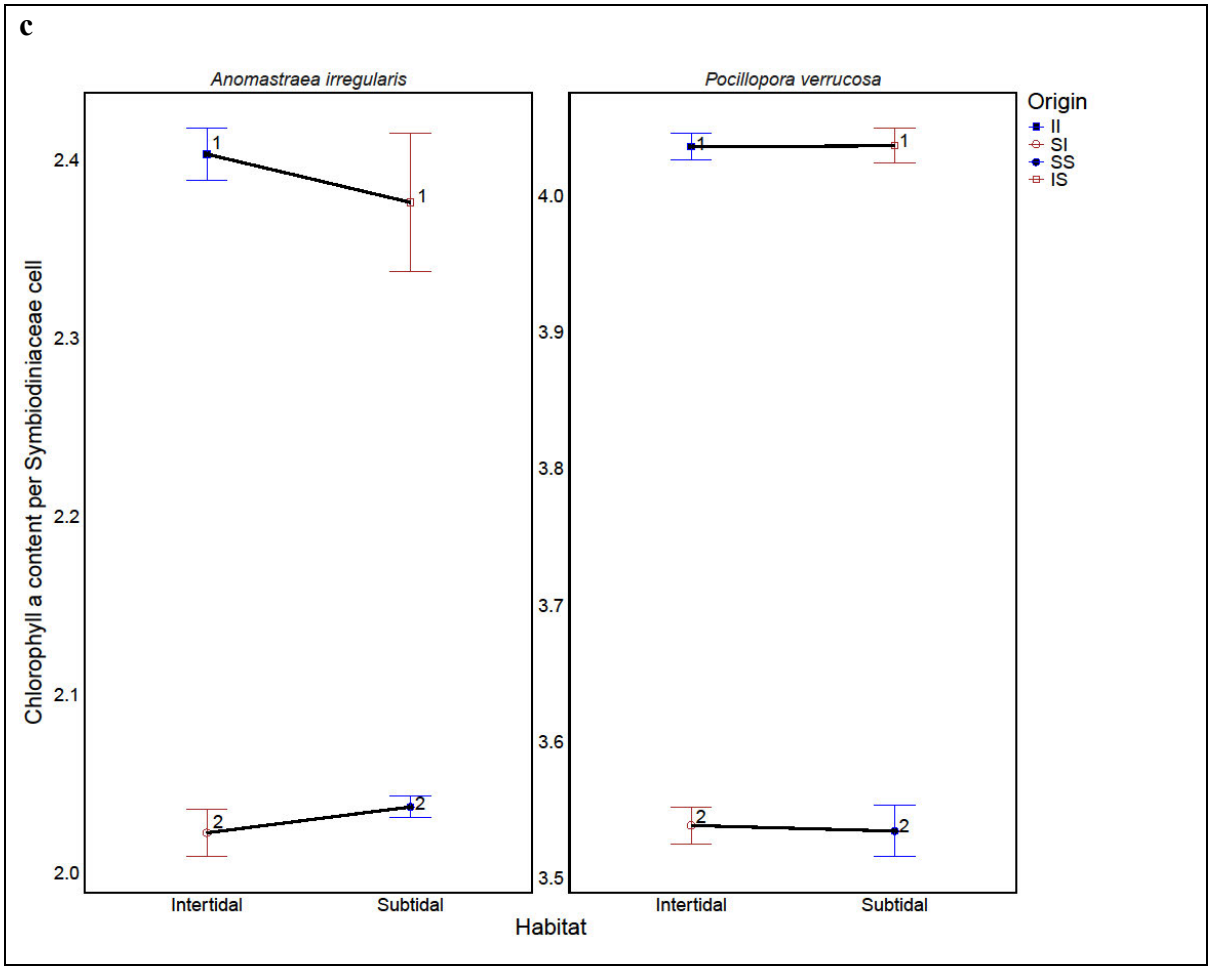
in the intertidal and subtidal habitats at the start and end of the experiment. Numbers (1, 2, 3, 4, and 5) indicate significant differences amongst tested groups (II, SS, IS, and SI) for each species according to Bonferroni pairwise tests for the GEEs.



b


Anomastraea irregularis
Pocillopora verrucosa
Origin
II
SI
SS
IS
Chlorophyll a content (µg cm^{-2})
1.4
1.3
1.2
1.9
1.8
1.7
1.6
Intertidal
Subtidal
Habitat

c


Anomastraea irregularis
Pocillopora verrucosa
Origin
II
SI
SS
IS
Chlorophyll a content per Symbiodiniaceae cell
2.4
2.3
2.2
2.1
2.0
4.0
3.9
3.8
3.7
3.6
3.5
Intertidal
Subtidal
Habitat

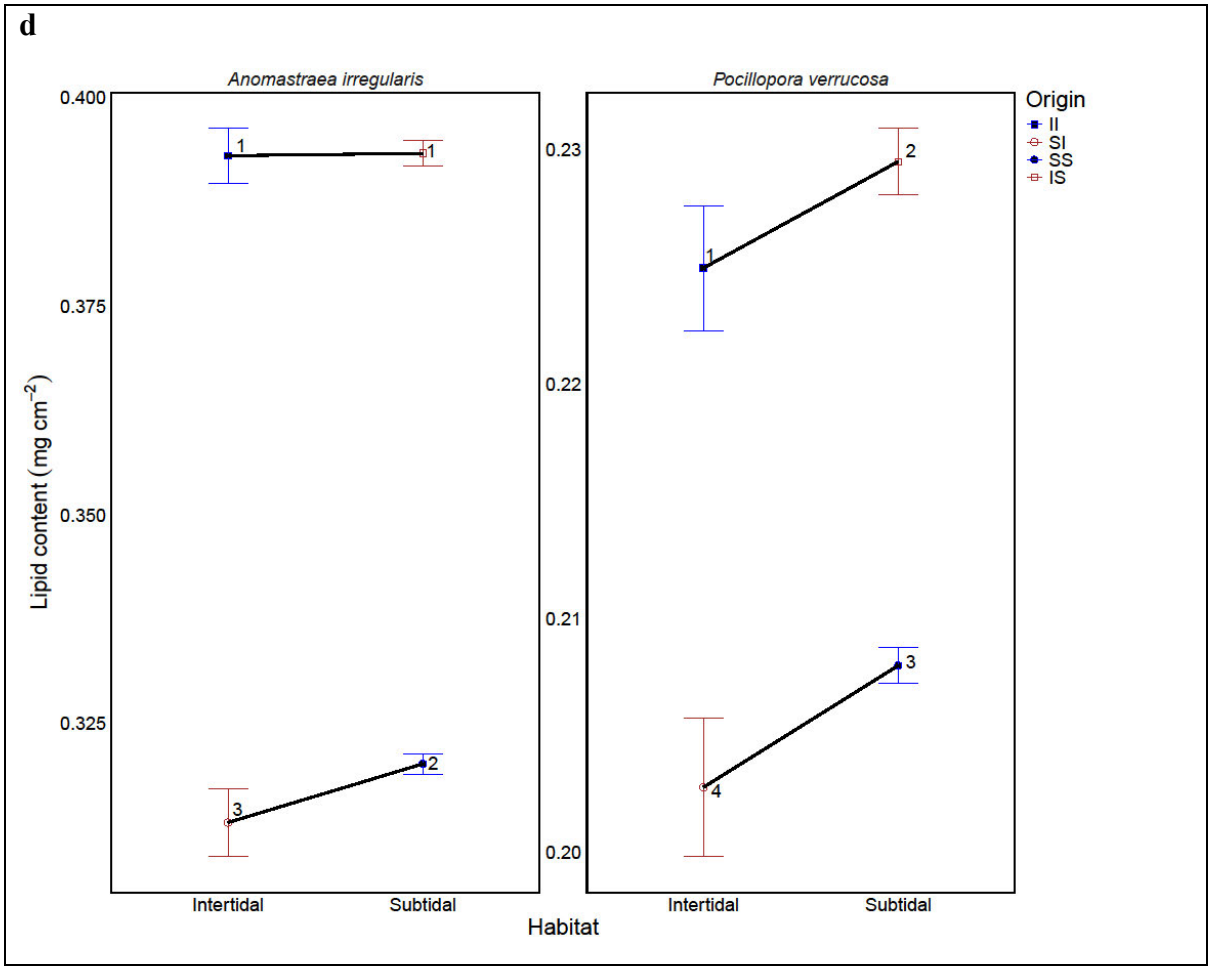


Figure 5.7: Reaction norms showing the overall **a** Average Symbiodiniaceae cell density (number of cells. cm^{-2}) ± 1 standard error, **b** Average chlorophyll-a concentration (µg. cm^{-2}) ± 1 standard error, **c** Average chlorophyll per Symbiodiniaceae cell ± 1 standard error, and **d** Average lipid concentration (mg. cm^{-2}) ± 1 standard error in *Anomastraea irregularis* and *Pocillopora verrucosa* fragments transplanted in the intertidal and subtidal habitats. Numbers (1, 2, 3, and 4) indicate significant differences amongst tested groups (II, SS, IS, and SI) for each species according to Bonferroni pairwise tests for the GEEs.

5.5 Discussion

This study adds to the limited knowledge of how high-latitude corals of different morphology and of habitats of differing environmental regimes react physiologically to new environmental conditions. The health and physiological rates of the native fragments of both species in both habitats remained constant throughout the study (**Figure 5.2 and 5.3**) showing that handling had negligible effect on the corals and that the results of the transplantation between the two habitats were in fact due to the environmental changes experienced. Physiological analyses of

the intertidal and subtidal corals of the two species exposed to the same environmental conditions revealed dynamic and divergent responses. This suggests that some adaptive differences exist between these coral populations (Kenkel and Matz 2016; Tisthammer et al. 2021; Jacquemont et al. 2022; McRae et al. 2022). Overall, the physiological responses indicated more resilient traits in the intertidal corals. Differences in mortality, P:R ratio (**Figure 5.4**), and tissue composition (**Figure 5.7**) showed that intertidal corals were more physiologically plastic, making them more resilient to the variable intertidal habitat. Generally, the reaction norms showed that the intertidal fragments of both species were able to adjust their physiology and acclimatise to the subtidal habitat, but the subtidal fragments did not fare as well in the intertidal habitat. Conversely, the subtidal fragments of both species showed local adaptation to the benign subtidal habitat and did not perform well in the dynamic intertidal habitat. The two species also responded differently, concurring with literature that coral physiological responses to environmental stressors are diverse and not easily predictable and are important for their resilience (Camp et al. 2018b; Jacquemont et al. 2022). However, except for temperature no other environmental variable (such as light, ultraviolet-irradiance, waterflow, pH, dissolved oxygen) was measured during this study. These factors can influence the thermal tolerance/susceptibility of corals (Brown 1997; McClanahan et al. 2005; Brown and Dunne 2016). It is quite likely that these environmental parameters may have varied throughout the study especially between the two habitats. It is therefore noteworthy that the results of this study must be conservatively interpreted because the nature of *in situ* studies makes them vulnerable to unforeseen environmental variability (Mayfield et al. 2012).

Published literature on the *in situ* physiological rates of these two high-latitude coral species in this region is scant. Respiration rates usually rapidly change when corals are stressed and can indicate sub-lethal stress responses (Lesser 2013). There seemed to be different physiological strategies employed by the two species with *Anomastraea irregularis* having changed its respiration rates while *Pocillopora verrucosa* had no change when transplanted between the two habitats (**Figure 5.4a**). Usually, an increase or decrease in coral respiration rates occurs when there are changes in the environmental conditions, while no change would indicate acclimatisation or no stress response (Guppy and Withers 1999; Sawall et al. 2011; Osinga et al. 2012). For example, *P. verrucosa* in Saudi Arabia rapidly altered their metabolic rates when transplanted between oligotrophic offshore to eutrophic nearshore habitats (Beisiegel 2012) and *P. aliciae* in the temperate reefs of south-eastern Australia was also able to adjust its metabolic rates to withstand exposure to short-term increased temperature (González-Pech et

al. 2022). *Pocillopora* is a cosmopolitan genus and are known as hardy 'weedy' or 'tramp' genera with varying physiological responses to environmental stressors (Veron and Stafford-Smith 2000) but are usually not considered as thermo-tolerant genera (Guest et al. 2016; Pisapia et al. 2016). The nonsignificant difference in respiration rates of the foreign *P. verrucosa* fragments could imply that the species were within their environmental threshold or that there is a lack of ability to adjust their rates when stressed. The lack of significant change in the respiration rates of the foreign *P. verrucosa* fragments could have meant that there was no increase in metabolic activities to mediate any environmental stress when transplanted, which may have led to the much lower coral health scores of foreign *P. verrucosa* fragments (**Figure 5.2**) and the mortalities of the SI *P. verrucosa* fragments (**Appendix 20**).

The reaction norm showed that SI *A. irregularis* fragments had significantly higher respiration rates than the native fragments of both habitats (**Figure 5.4a, Table 5.1, Appendix 22**). Furthermore, the II *A. irregularis* fragments had significantly higher respiration rates than the SS fragments (**Figure 5.4a, Table 5.1, Appendix 22**). An increase in respiration rates suggests that there was an increase in the metabolic activity of the corals (Anderson et al. 2019). The SI *A. irregularis* fragments may have been trying to mediate the change in environmental conditions, while the II fragments may have adapted a higher metabolic rate than the SS conspecifics to cope with the constant high fluctuating conditions (Kenkel et al. 2015). The reaction norm also showed that IS *A. irregularis* had similar respiration rates to the SS *A. irregularis* fragments (**Figure 5.4a**), which highlights the ability of intertidal *A. irregularis* to quickly acclimatise to the more benign subtidal habitat. During a year-long study Kenkel and Matz (2016) found similar results with inshore *Porites astreoides* acclimatising to offshore conditions and that the inshore transplants could outperform the native offshore corals.

The lower photosynthetic rates and resultant P:R ratios in the SI fragments (**Figure 5.4b**) may have been because the high light conditions in the intertidal habitat likely pushed the lower light-adapted Symbiodiniaceae in these fragments to photoinhibition or even caused photodamage (Yap et al. 1998; Ralph et al. 2005). Conversely, the Symbiodiniaceae in the IS fragments may have attained photoacclimation to the subtidal habitat as they had similar gross photosynthetic rates and resultant P:R ratios to the SS fragments (**Figure 5.4b**). The P:R ratio reflects the amount of energy a coral receives from its Symbiodiniaceae, with a ratio greater than one indicating sufficient energy from the Symbiodiniaceae while a ratio less than one indicates insufficient energy from their Symbiodiniaceae (Muscatine et al. 1981). The P:R ratios of the native and the foreign *P. verrucosa* fragments remained above one during this

study, suggesting that the Symbiodiniaceae in the fragments still produced enough energy for the corals even with the stress of altered environmental conditions. However, the SI *P. verrucosa* fragments could have been receiving low-quality photosynthates from their endosymbionts (Tremblay et al. 2016), which may have reduced their ability to acclimatise to the new habitat, which would have caused a further decline in the coral's health (**Figure 5.2**) and led to their deaths (**Appendix 20**). Ziegler et al. (2014) showed in an *in-situ* experiment that *P. verrucosa* from the Red Sea had limits to physiological plasticity when transplanted to different depths.

Despite being in healthy conditions (**Figure 5.2**) the native and foreign *A. irregularis* fragments in both habitats had P:R ratios below one, indicating that the Symbiodiniaceae in the fragments were not producing enough energy for the corals. A P:R ratio less than 1 is usually seen in severely bleached corals or corals that experience turbid conditions (Grottoli et al. 2004; Browne et al. 2014). Smit (2014) found that heterotrophy contributes more to the metabolism of *A. irregularis* than *P. verrucosa* through differences in the $\delta^{13}C$ and $\delta^{15}N$ isotopes between coral host tissue and Symbiodiniaceae cells. Furthermore Boodraj and Glassom (2022) found the ingestion rates of *A. irregularis* were much higher than *P. verrucosa*. These studies provide evidence that *A. irregularis* may depend more on heterotrophy than photosynthesis to meet its metabolic needs, which may explain the unusually low P:R ratios even under seemingly non-stressful conditions. The difference in P:R ratios between the species could, therefore, be explained by their heterotrophic/autotrophic dependence on meeting their metabolic requirements. In addition, the higher surface area and thinner tissue of *P. verrucosa* would allow for higher photosynthetic rates than *A. irregularis* (Edmunds and Gates 2002; Osinga et al. 2012; Conley and Hollander 2021).

Coral growth is an energy-consuming process that is sensitive to environmental changes (Anthony and Connolly 2004) and may vary with the climatological regime as well as acclimatisation or adaptation to local conditions (Dandan et al. 2015; Sawall et al. 2015; Barott et al. 2021). The growth rates of the native and foreign fragments of both species in both habitats increased from the start of the study and then dropped at the last sampling point but remained above the initial growth rates (**Figure 5.5a**). Coral growth may increase during summer months when environmental conditions are optimum for Symbiodiniaceae photosynthesis (Al-Hammady 2013). However, there is a threshold beyond which growth rates can drop when environmental parameter. The drop in growth rates at the end of the study could

have been due to the environmental conditions reaching suboptimum levels as it is the time of the year where light stress and freshwater inundation is highest in the region.

Overall, for *A. irregularis* the native intertidal fragments had lower growth rates than the native subtidal fragments (**Figure 5.5b and Appendix 23**). This is in contrast to literature that has shown corals found in shallow water intertidal habitats have higher growth rates than corals in the subtidal habitats, mainly because of the increased photosynthesis that can occur from the higher light conditions (Beisiegel 2012; Dandan et al. 2015; Tamir et al. 2020). However, too high temperature and light conditions can cause photodamage thereby reducing growth in corals (Sinutok et al. 2022). Despite increasing their initial growth rates, the foreign fragments of both species in both habitats had significantly lower growth rates than the native fragments during this study (**Figure 5.5b and Appendix 23**). Bay and Palumbi (2017) had a similar result where corals from a stressful environment had lower growth rates when transplanted to a more stable environment. It is believed that this depression in growth rates could be an inherent trade-off because energy needs to be diverted towards process required to survive in the sub-optimal environmental conditions (Bay and Palumbi 2017). Therefore, the acclimatisation of corals to extreme environments or new environments may come at the expense of growth. Reduced coral growth can negatively impact reef functioning and health (Harborne et al. 2017). However, Barott et al. (2021) showed that two coral species from extreme habitats in Hawaii were able to acclimatise to new reef environments without declines in growth, so there may be instances where coral resilience may not cause trade-offs to their fitness depending on the species and their prior exposure to environmental stress.

Changes in the tissue composition of corals in terms of the Symbiodiniaceae cell density, Symbiodiniaceae chlorophyll-a concentration, Symbiodiniaceae chlorophyll-a concentration per cell and lipid concentration of the corals can also explain a coral's tolerance/susceptibility to stressors due to their influence on the corals energy budget (Muscatine 1990; Nystrom et al. 2001; Houlbrèque et al. 2003). None of these properties in either species native fragments changed between the start and the end of the study except for the lipid content in *P. verrucosa* II fragments which was significantly lower at the end compared to the start of the experiment (**Figure 5.4**, **Appendix 24**, **27, 28, and 29**). This lack of change would suggest that the native corals were within their normal environmental thresholds. This may also tie into why there were no significant change in the respiration and photosynthetic rates, P:R ratios during this study. The native intertidal *P. verrucosa exhibited* changes, potentially using lipids for energy. A decrease in lipids could have also resulted from damage through lipid peroxidation from the

production of reactive oxygen species due to thermal and/or light stress which would be prevalent in the intertidal habitat (Lesser 1997; Downs et al. 2002; Rodrigues and Grottoli 2007). Smit (2014) found that lipids declined in *P. verrucosa* between austral summer and autumn and stayed low until spring, suggesting that low light in winter might be more stressful than the elevated summer temperatures.

There seemed to be an inherent species and habitat difference in the tissue composition of the native fragments (**Figure 5.7**). In both species, the Symbiodiniaceae cell density, Symbiodiniaceae chlorophyll-a concentration, and Symbiodiniaceae chlorophyll-a concentration per cell were lower in the subtidal fragments, which could be explained by the lower light conditions that prevail in the subtidal habitat. The lower light conditions usually cause a reduction in Symbiodiniaceae so that self-shading is reduced, therefore maximising the photosynthetic potential of the Symbiodiniaceae in lower light conditions (Jones 1997). Usually, higher lipid concentrations are associated with stress-tolerant corals (Grottoli et al. 2014; Sawall et al. 2015; Schoepf et al. 2015a; McLachlan et al. 2022) as it is used as an energy source to sustain metabolic processes during bleaching events (Rodrigues and Grottoli 2007). This suggests that the intertidal corals in this study may possess higher lipid concentrations than the subtidal conspecifics as an adaptive strategy to mediate the metabolic demand that is involved with persisting in a highly fluctuating environmental condition. The higher chlorophyll-a per Symbiodiniaceae cell in the intertidal fragments would also suggest stress tolerance, as it is known to aid coral persistence in extreme habitats (Camp et al. 2016b).

Overall, the Symbiodiniaceae cell density and lipid concentration was higher in the *A. irregularis* fragments, while the chlorophyll-a concentration and chlorophyll-a concentration per Symbiodiniaceae cell were higher in the *P. verrucosa* fragments (**Figure 5.4**). The higher Symbiodiniaceae cell density in *A. irregularis* may be a result of its thicker tissue, which provides the cells with more efficient self-shading, thereby providing protection from light stress (Hoegh-Guldberg 1999; Loya et al. 2001; Thornhill et al. 2011). The higher lipid concentrations in *A. irregularis* than *P. verrucosa* may be a result of increased heterotrophy in *A. irregularis* over *P. verrucosa* (Houlbrèque and Ferrier-Pagès 2009; Smit 2014). Ziegler et al. (2014) indicated that *P. verrucosa* does not have heterotrophic plasticity, so heterotrophy may not play a role in regulating its tissue composition. Higher chlorophyll-a concentration and chlorophyll-a concentration per cell in *P. verrucosa* may be a strategy to maximise photosynthesis and attain more energy from fewer cells (Le Tissier and Brown 1996; Jones 1997).

For both species, the transplantation between habitats affected their tissue composition (**Figure 5.7**). The ability of a coral to regulate the Symbiodiniaceae cell density was also believed to be due to its skeleton being perforate/imperforate (Kinzie et al. 1984). Ziegler et al. (2014) showed that *P. verrucosa*, an imperforate species, displayed lower tissue volumes and was unable to regulate Symbiodiniaceae cell density. Therefore, it is possible that higher light and temperature in the intertidal habitat caused the reduction of the Symbiodiniaceae cell density, chlorophyll-a concentration, and chlorophyll-a concentration per Symbiodiniaceae cell in SI *P. verrucosa* fragments at the end of the experiment as compared to the initial density (Muscatine 1990; Nystrom et al. 2001; Houlbrèque et al. 2003). Contrastingly, SI *A. irregularis* fragments did not experience a reduction in the Symbiodiniaceae cell density, chlorophyll-a concentration, and chlorophyll-a concentration per Symbiodiniaceae cell at the end of the experiment. This could be explained by the lower lipid concentration also seen in these fragments; they could have been using the energy to maintain the tissue composition and counteract the stress of the intertidal environment, which was seen with their higher respiration rates (Anderson et al. 2019; González-Pech et al. 2022). The lower lipid concentration in the SI *P. verrucosa* fragments at the end of the experiment could have also been a result of breakdown to use as an energy source or through lipid peroxidation due to oxidative stress as a result of the thermal and light stress (Lesser 1997; Downs et al. 2002; Rodrigues and Grottoli 2007; Smit 2014).

The IS *A. irregularis* fragments had significantly lower Symbiodiniaceae cell density and chlorophyll-a concentration at the end of the experiment as compared to the initial density, which could have resulted to maximise photosynthesis in the lower light conditions (Jones 1997). The chlorophyll-a concentration per Symbiodiniaceae cell in these fragments did not significantly differ between the start and the end of the experiment, and this could be because of the reduced competition for nutrients since there were fewer Symbiodiniaceae cells (Jones 1997). An increase in chlorophyll-a per Symbiodiniaceae cell could also be more energy efficient for the coral and/or the Symbiodiniaceae than to procure Symbiodiniaceae cells (Rodrigues and Grottoli 2007). The Symbiodiniaceae cell density, chlorophyll-a concentration, and chlorophyll-a concentration per Symbiodiniaceae cell of IS *P. verrucosa* fragments did not significantly differ between the start and end of the experiment, indicating acclimation to the subtidal habitat. No significant difference in the lipid concentration of the IS fragments of both species at the end of the experiment indicated that they maintained their energy reserve throughout the study, which further suggested that the subtidal habitat conditions were not

significantly stressful. Other studies have hypothesised that transplanted corals had increased tissue lipid contents due to increased heterotrophic and phototrophic nutrition (Beisiegel 2012; Seemann et al. 2013; Tisthammer et al. 2021). The photosynthetic rates of these fragments did not significantly increase; heterotrophy was not measured in this study and, therefore, cannot be ruled out as contributing to the maintained lipid concentrations. Overall, the tissue composition results of this study highlight the importance of lipid concentration and the ability of the coral to manipulate their Symbiodiniaceae cell density/chlorophyll-a concentration to maintain energy balance when faced with varying environmental conditions.

From a physiological perspective, thermal stress is believed to induce bleaching by reducing the amount of light stress needed for photoinhibition (Hoegh-Guldberg 1999; Kramer et al. 2022). Therefore, light and temperature levels should be included when evaluating the bleaching thresholds of corals (Mason et al. 2020). However, photosynthetic active radiation was not measured during this study, which may have affected the bleaching of the corals, especially in the intertidal habitat. Despite lower coral health scores in the foreign fragments (**Figure 5.2**), the health scores were above 3.6 which is considered still healthy (Schoepf et al. 2020). Both the duration and intensity of thermal stress dictate bleaching events (Carballo-Bolaños et al. 2019). The degree heating weeks over the course of the study remained below 4°C-weeks or greater (**Figure 5.1c**)and therefore it was within the corals' normal physiological threshold (Liu et al. 2014). This could explain the lack of significant bleaching witnessed in this study. Barott et al. (2021) had similar results of corals from extreme habitats attaining acclimatisation to new environmental conditions during a nonbleaching year and indicated that if these corals are transplanted to new reefs during bleaching years, then the population fitness of those reefs would be enhanced.

Approximately 80% of corals procure their Symbiodiniaceae from their surrounding environment (Nitschke et al. 2015). The differences in the photosynthetic rates between the species and habitats and differences in growth rates between habitats could also be explained by different Symbiodiniaceae taxa being housed in the tissue of the corals (Baker 2003; Cunning et al. 2015). This study did not identify the genetic identity of Symbiodiniaceae housed by the corals. However, a previous unpublished local study on these species showed that both species from both habitats housed the same clade C Symbiodiniaceae (S Ntuli personal communication). Therefore, it is possible that the Symbiodiniaceae type did not significantly influence the differences in the photosynthetic rates between the species and habitats. A study by Turnham et al. (2023) in the Eastern Pacific Ocean also showed that there

were no significant physiological differences in *P. grandis* colonies hosting the more thermally tolerant Symbiodiniaceae *Durusdinium glynnii*, further indicating that thermally tolerant coral host-symbiont combinations can persist during thermal stress without considerable physiological trade-offs. However, even if both species harbour the same clade C Symbiodiniaceae, there may nevertheless be habitat-specific differences on the species level which can influence physiological activities (Stat et al. 2008).

Thick-tissue massive corals that are exposed to extreme spatial and temporal physiochemical shifts are believed to become preconditioned to withstand environmental disturbances like increased thermal stress (Putnam et al. 2017). The results of this study showed that the thick-tissue coral *A. irregularis* from the intertidal habitat appeared to be more tolerant to environmental changes than the thin-tissue coral *P. verrucosa*. Tamir et al. (2020) similarly found coral morphology and niche to influence survival and performance after a year-long reciprocal transplant experiment between a shallow marginal habitat and a deeper stable habitat in Eilat, Israel. The morphology of corals influences their interactions with the physical environment (Zawada et al. 2019a). This has implications for future reefs since following marine heatwaves, communities of thermally tolerant corals become dominant over sensitive species, which die off and, in turn, the reef's resilience increases (McClanahan et al. 2007; Baum et al. 2023). However, the complexity of the reefs may change since slow-growing massive species may dominate over fast-growing branching species, which would reduce the reef structures (Baum et al. 2023). Moreover, the increased frequency, duration, and intensity of thermal anomalies may render the tolerant corals impotent (Baum et al. 2023).

Jacquemont et al. (2022) indicated that no single physiological response can explain coral tolerance to thermal stress and that various mechanisms will be responsible. For example, genetic and molecular changes can also contribute to the tolerance/susceptibility of corals to environmental stressors (Barshis et al. 2010; Dziedzic et al. 2019). Further investigation into how long the resilience of the transplanted fragments can be maintained is also required to understand if the transplanted corals can be used in restoration initiatives. Schoepf et al. (2019) showed that corals from thermally extreme reefs can be used to restore cooler, degraded reefs since the corals could maintain thermal tolerance in more stable temperatures for over nine months. However, it is noteworthy that acclimation to dynamic habitats may not enhance physiological tolerance of some corals (Camp et al. 2016a) and that the thermal tolerances of corals from extreme habitats may also be rigid due to genetics and may not exceed temperature increases over 1°C above the threshold, which means these naturally tolerant corals may not

be able to withstand the ongoing unprecedented climate change (Schoepf et al. 2015b; Baums et al. 2019; Schoepf et al. 2019). In fact, Le Nohaic et al. (2017) reported that naturally heat-tolerant corals from extreme thermally variable habitats in Australia were vulnerable to a heatwave that occurred in 2015-2016. Furthermore, expending energy to resist thermal stress may increase their sensitivity to future increased thermal stress and also make them vulnerable to diseases or predators (Grottoli et al. 2014). The corals' reproductive success may also be lowered due to the energy spent dealing with thermal stress (Ward et al. 2002). Several other local stressors (e.g. eutrophication, overfishing, coastal development, and pollution) could act in synergy with thermal stress, which may also lower the corals' resilience (Carilli et al. 2009; Baum et al. 2023). The ability of the corals to recover (rate of repair or Symbiodiniaceae recolonisation of coral tissue) from stressors also needs to be studied since recovery is an important factor in resilience (Mason et al. 2020).

Ultimately, this study showed that the physiological responses can vary between species and habitats when exposed to new environmental conditions. Overall, the hypothesis for this study was accepted since the intertidal corals and the massive species *A. irregularis* seemed more physiologically plastic; they did not die when transplanted in the subtidal habitat, and their physiological rates were generally not negatively impacted. Conversely, the subtidal corals transplanted to the intertidal habitat experienced mortalities and lower health scores. The degree of physiological plasticity in the intertidal fragments indicates that environmental variability in the intertidal habitat may be driving their thermal resilience. Studies have shown that corals from habitats with environmental variability can have local adaptations as well as physiological plasticity to thrive in the heterogenous conditions and that sometimes their physiological plasticity means that they can acclimatise to new environments that do not match their origin conditions (Thomas et al. 2018; Kurihara et al. 2021; Marhoefer et al. 2021). The subtidal corals seemed to have fixed genetic effects to the stable subtidal environment and limited physiological plasticity therefore did not perform well when transplanted. Local adaptation has been observed in corals from stable environments because specific traits develop in these corals to enhance their fitness relative to their local conditions but this limits their ability to adjust to new environmental regimes (Castillo et al. 2024). The subtidal and intertidal habitats at Tressure Beach are periodically connected during spring hightide and Chiazzari et al. (2013) showed genetic overlap of *Acropora tenuis* between the two habitats but there has been no genetic study on these species to confirm genotypes. However, it would appear that the stable conditions of the subtidal habitat and dynamic conditions of the intertidal habitat

drives origin effects seen in the distinct physiological advantages highlighted in the reaction norms for the native subtidal and intertidal fragments (Kawecki and Ebert 2004). Future studies should look at genetics of the corals and the Symbiodiniaceae to be better informed for conservation initiative. There is potential for the resilient corals of the intertidal habitat to be used for further conservation initiatives because of their seemingly quick acclimatisation to new environmental conditions (Morikawa and Palumbi 2019; Barott et al. 2021). However, transplanting resilient corals to impacted reefs needs to be done with caution to prevent carrying disease or other unwanted species to wreak havoc on already vulnerable ecosystems (Baums et al. 2019). Additionally, it is noteworthy that the thermal tolerance of the intertidal corals could decline in cooler, less stressful environments (Howells et al. 2013). The subtidal corals which show strong local adaptation could be used in restoration efforts, but they need to be transplanted to matching environmental conditions because they are adapted to thrive under specific environmental parameters (Scucchia et al. 2023).

5.6 Conclusion

This study explored the natural tolerance/susceptibility of two species of differing morphologies from two different habitats with contrasting environmental conditions. The reciprocal transplant results highlighted physiological differences in stress responses between the intertidal and subtidal corals and between species. The physiological characteristics indicated more stress resilient traits (metabolic rates and tissue composition) of intertidal corals to deal with the dynamic intertidal conditions. The results of this study highlight the importance of investigating and conserving corals from extreme habitats since they may possess favourable stress-tolerant traits that are required for the persistence of coral reefs under the rapidly changing global climate. The intertidal corals' acclimatisation to the subtidal habitat occurred quickly and some of their physiological rates matched the native subtidal corals during the study despite decreases in growth rate. The subtidal corals physiological rates and tissue composition did not fare well in the intertidal habitat and there were mortalities early in the study. Six months may not be sufficient for transplants to fully acclimatise to novel environmental conditions. The thick-tissue massive species *Anomastraea irregularis* appeared more physiologically plastic and, therefore, tolerant than the thin-tissue branching species *Pocillopora verrucosa* to the changes in environmental conditions. Physiologically plastic corals seem to have more tolerance to changes in environmental conditions and can be used in further conservation initiatives.

Chapter 6: General discussion/recommendations and conclusion

6.1 General discussion

Climate change and its effects on coral reefs have now reached unprecedented territory (Hoegh-Guldberg et al. 2023; Reimer et al. 2024). The year 2023 had record-breaking anomalously high sea surface temperatures globally, and coupled with climate-change amplified El Niño conditions, mass bleaching events were predicted to occur in 2024 (Hoegh-Guldberg et al. 2023). NOAA (2024) confirmed the 4th global coral bleaching event which started in the Northern Hemisphere summer of 2023 and followed the Southern Hemisphere summer of 2023-2024. This mass bleaching event marks the second event in the last decade (Reimer et al. 2024). Steadily increasing sea surface temperatures and stronger El Niño events are predicted to intensify these mass bleaching events if current carbon dioxide emissions continue unchecked (Hoegh-Guldberg et al. 2023). However, laboratory and field studies conducted over the years show that corals have differential resilience/susceptibility to thermal stress (Kuffner et al. 2015; Chapron et al. 2022; Brown et al. 2023; Hoegh-Guldberg et al. 2023; Kuo et al. 2023).

Compared to traditional tropical low-latitude reefs, high-latitude corals of South Africa exist in habitats of varying environmental regimes and have not faced major bleaching despite ocean warming trends (Schleyer et al. 2018). This is thought to be an important factor conferring a natural resilience to predicted warming. Therefore, this thesis focused on understanding the physiological mechanisms that drive resilience/susceptibility to prolonged thermal stress in the understudied high-latitude natural stock corals of two different species and morphology from an environmentally variable habitat and a moderate habitat. This thesis provides a comprehensive account of the physiological mechanisms used by corals from marginal and extreme habitats to survive thermal stress thereby contributing fundamental knowledge that can aid conservation initiatives and support the long-term persistence of coral reefs.

Chapter 2 and 3 showed that prolonged exposure to temperatures above the corals' environmental average for three months caused differential bleaching and deaths due to their ability to adjust physiologically and mediate oxidative stress. The usefulness of the flexi-chamber and three-dimensional photogrammetry methods to measure physiology of small fragments was assessed and optimised in Chapter 4 so that the physiological rates of fragments could be measured in the six-month reciprocal transplant study in Chapter 5. Collectively, the results of this thesis highlighted the inherent physiological differences that exist between the corals of different species in different habitats. Through the long-term laboratory and field

studies, variable thermal regimes and coral species influenced coral resilience to thermal stress. Insight into the possible physiological mechanisms contributing to the resilience of the intertidal corals and *Anomastraea irregularis* was provided in this thesis.

Chapter 2 reported how prolonged exposure to the conservative and high-end of predicted increase in temperatures on the east coast of South Africa affects the corals' physiological rates and biochemical activity. The findings of this study demonstrated that high-latitude corals can endure substantial thermal stress, surviving >8°C-weeks. The thermal resilience was not uniform across species or habitats, as subtidal corals and *Pocillopora verrucosa* exhibited greater susceptibility, experiencing greater mortality under the prolonged heat exposure. This variability highlights the importance of species- and habitat-specific physiological mechanisms in shaping coral resilience to thermal stress. While high-latitude reefs may collectively possess an enhanced capacity to withstand warming, certain coral populations and species may be better equipped to cope with increased thermal stress than others. Physiological plasticity and maintenance of higher Symbiodiniaceae densities, chlorophyll-a, and lipid concentrations were found to cause increased resilience to thermal stress in intertidal corals compared to their subtidal conspecifics. The resilience of *A. irregularis* compared to *Pocillopora verrucosa* was believed to have resulted from the thicker tissue, which permitted higher Symbiodiniaceae density and lipid content, and lower P:R ratios, which indicated a more heterotrophic nature. The main findings from the experiment concur with some of the literature (Oliver and Palumbi 2011; Kenkel and Matz 2016; Tkachenko and Soong 2017; Tisthammer et al. 2021; Jacquemont et al. 2022; McRae et al. 2022; Speelman et al. 2023) and reiterates that differential responses to thermal stress are influenced by innate physiological responses due to thermal regimes and species.

Chapter 3 further detailed the biochemical activity of the corals that were exposed to thermal stress in Chapter 2. The thermal stress treatments caused higher protein concentrations and antioxidant enzyme activities (superoxide dismutase, catalase, and glutathione peroxidase) in both species from both habitats. The results are indicative of higher oxidative stress experienced with thermal stress, which corresponds with the oxidative bleaching theory (Lesser 1997; Downs et al. 2002; Weis 2008; Wall et al. 2021). Furthermore, thermal regimes of habitats and coral species were also found to influence antioxidant enzyme activities. Higher protein concentrations and lower antioxidant enzyme activities were evident in the more resilient intertidal fragments than subtidal fragments and *A. irregularis* than *P. verrucosa* when thermally stressed. The higher protein concentrations in the intertidal fragments and *A.*

irregularis were thought to assist physiological processes, thereby making them more resilient to thermal stress. This meant lower oxidative stress and, therefore, lower antioxidant activities than the sensitive corals. Some literature has similarly found sensitive corals enhance antioxidant activities due to increased oxidative stress experienced with increased thermal stress (Downs et al. 2002; Griffin et al. 2006; Krueger et al. 2015; Dias et al. 2019a; Huang et al. 2024).

The laboratory experiment highlighted the physiological mechanisms responsible for thermal resilience in the corals. However, the static nature of laboratory experiments cannot account for the dynamic environmental conditions corals encounter *in situ*. A way to decipher whether the resilience of the intertidal are due to adaptation or acclimation to the variable conditions would be to perform a reciprocal transplant experiment (Kawecki and Ebert 2004). Therefore, Chapter 5 was an important aspect of this thesis, investigating the thermal resilience of the corals *in situ* by reciprocally transplanting the corals between the habitats during the austral spring and summer months. Like the laboratory results, intertidal and *A*. *irregularis* fragments were found to be more resilient. Physiological plasticity appeared to contribute to thermal resilience. Intertidal fragments were able to adjust their P:R ratios and maintain higher Symbiodiniaceae cell density, Symbiodiniaceae chlorophyll-a concentration, Symbiodiniaceae chlorophyll-a concentration per cell, and lipid content. *Anomastraea irregularis* appeared more physiologically plastic, altering respiration rates, and maintaining higher Symbiodiniaceae cell density and lipid concentration.

High-latitude corals have been increasingly studied for their potential resilience to climate change (Keshavmurthy et al. 2021; Schoepf et al. 2023). Low-latitude reefs have historically been the focus of coral bleaching research and are known to experience low level bleaching when there is greater than 4°C-week and severe bleaching when it is greater than 8°C-week (Hughes et al. 2003; Hoegh-Guldberg et al. 2018). The results from this study show that collectively the high-latitude corals of South Africa studied here are far more resilient than the low-latitude reefs persisting through >8°C, although with differential resilience. South Africa may thus serve as future climate refugia. It is possible that the corals studied here may be more resilient and poses traits to deal with the predicted warmer oceans. Furthermore, similar to other extreme habitats (Barshis et al. 2018; Camp et al. 2018a; Schoepf et al. 2020; Scucchia et al. 2023) the intertidal pools seem to be local resilience hotspots that are naturally preconditioning the corals to extreme conditions and may be beneficial to the corals towards climate change. These tide pools abundantly occur along the east coast of South Africa and do

house a variety of hard coral species (Smit 2014; Smit and Glassom 2017). Chiazzari et al. (2013) showed genetic overlap of *Acropora tenuis* between the intertidal and subtidal habitats, which means that the heat-resistant coral populations in the intertidal pools may be important for South African corals reefs especially in light of predicted climate change.

6.2 Limitations and recommendations

To minimise the impact on coral populations, fragments were used in the experiments to allow for more replicate measurements across treatments with the same amount of source material (Shafir et al. 2003; Shafir et al. 2006). When studying branching species, fragments from colonies should ideally be placed close together to create microenvironments between branches, thereby mimicking the complexity of the natural colony (Edmunds et al. 2022). This was not done in the experiments; only a single fragment of *P. verrucosa* from each colony was used. Future studies that use fragments from branching colonies should account for the structural complexity by placing fragments close together to attain more accurate results.

Symbiodiniaceae genotypes like *Durusdinium* spp. and *Cladocopium* spp. can influence coral resilience (Berkelmans and van Oppen 2006; Stat and Gates 2011; Levas et al. 2013; Hume et al. 2020). Intraspecific variation in thermal stress responses has also been noted among colonies of some coral species, and it is an important factor that contributes to thermal resilience (Kavousi et al. 2020). It is noteworthy that there were no significant colony effects in the laboratory and *in situ* studies for any of the physiological factors tested in this thesis. No genetic analyses were conducted on the corals or their Symbiodiniaceae to determine specific genotypes in the laboratory and *in situ* studies. There is a possibility that the lack of colony effects could mean that the colonies were not genetically distinct. The strong selective pressures in extreme habitats such as mangroves were previously found to decrease intrapopulation genetic diversity (Scucchia et al. 2023). Further analysis of the genetic stock of the corals and their Symbiodiniaceae is required to identify the genotypes present and to understand the role genetic variability plays in the thermal resilience of these corals, especially genetic divergence between habitats. Lower genetic diversity can compromise future competitive fitness and needs to be considered when utilising these resilient corals for conservation initiatives (Scucchia et al. 2023). The genotype of the coral host may limit the physiological responses of resilient corals when presented with new environmental conditions (Barshis et al. 2010). Therefore, determining how heritable the host and symbiont heat resistance considering new conditions is also important for understanding the long-term persistence of thermally tolerant corals (Barshis et al. 2010; Kurihara et al. 2021). Furthermore,

thermal tolerance can decline when corals from extreme environments are transplanted to less stressful environments (Howells et al. 2013). Therefore, it will be beneficial to further understand the persistence of the thermal tolerances and acclimatisation capacity of the corals studied in here. This can be achieved by conducting a similar heat stress experiment as in Chapter 2 (or repeating heat stress experiment) after the reciprocal transplant experiment as in Chapter 5.

This thesis also did not take other symbionts, such as bacteria, viruses, and fungi, into account when investigating the thermal resilience of the corals. Microbial communities of corals have been found to play a significant role in resilience to changing environmental conditions (Chan et al. 2024) and future climate change (Price et al. 2023). Future studies must account for other symbionts contributing to the resilience of the coral to climate change to fully understand the effects of climate change on the holobiont (Price et al. 2023).

The massive species *A. irregularis* was found to be more resilient compared to *P. verrucosa*, which has been seen in other thermal stress studies (Stimson et al. 2002; Bay et al. 2016; Pisapia et al. 2016). Literature on *A. irregularis* is scant and this thesis novelly highlighted its unique physiology and the potential importance of a mixotrophic strategy for thermal resilience. This shows that cataloguing responses of understudied species is very important to advance our understanding of coral thermal resilience. Autotrophic corals are predicted to lose their competitive advantage with increased thermal stress and will be the first to disappear from coral reefs (Conti-Jerpe et al. 2020) which means that mixotrophic species like *A. irregularis* may then dominate. This can have implications for reef functioning since branching families like pocilloporids and acroporids typically contribute significantly to the complex 3D frameworks of reefs and the carbonate cycle (Gilmour et al. 2013; Pratchett et al. 2015; Lange et al. 2020; Tortolero-Langarica et al. 2022). However, massive species have been found to also contribute to the long-term reef carbonate production and complexity significantly in shallow and deep coral reefs (Tortolero-Langarica et al. 2022). Although the carbonate production of massive species is lower than branching species and therefore the carbonate budgets and ecological functionality will still be compromised with the loss of branching families (Tortolero-Langarica et al. 2022). Furthermore, reefs that suffer low species richness and functional redundancy could face lower resilience following disturbance events (McWilliam et al. 2018). Romero-Torres et al. (2020) has shown the importance of resilient intertidal *P. verrucosa* to repopulate degraded reefs in the eastern tropical pacific. The results of this thesis also show the potential of using the intertidal *P. verrucosa* to recolonise degraded

reefs. However Hughes et al. (2023) showed that a large percentage of warm-adapted corals die after being transplanted and that translocation of corals would only be feasible over relatively short distances similar to the coral larvae dispersal capacity. Therefore, rather than simply transplanting fragments of intertidal *P. verrucosa* and *A. irregularis* to other locations, more manipulative coral conservation interventions such as selective breeding to make resilient hybrids and genetic modifications (van Oppen et al. 2015; Quigley 2024) could possibly exploit these favourable genotypes.

The natural migration of thermally resilient larvae from extreme habitats and the associated distribution of heat-adapted alleles can contribute to the persistence of coral reefs under climate change (Morikawa and Palumbi 2019). Intertidal corals have the potential to act as brood stock for nearby degraded reefs and promote recruitment and recovery with thermally resilient genotypes (Jung et al. 2021). However, these resilient corals can be vulnerable to climate change (Brown et al. 2024) especially if other local stressors are not mitigated (Schoepf et al. 2019). Here, the high-latitude corals, especially the intertidal population, were found to be thermally resilient. The results of this thesis highlight that in addition to the management and protection of thermally sensitive coral populations, such measures must also include protection of extreme and marginal populations as they may possess naturally thermal tolerant traits that can allow them to survive increasing thermal stress. The lack of physiological and biochemical recovery after two months also indicated that long recovery times may be required for these corals. This is of concern since less time between bleaching events is predicted in the near future (Hoegh-Guldberg et al. 2023). The long-term effects of the resilience on the fitness of these corals also need to be considered (Bove et al. 2022). For example, a year after recovering from the 2020 mass-bleaching event, *Acropora millepora* in the Keppel Islands had a 21% decrease in population-level reproductive output (Briggs et al. 2024). The decrease in reproductive output is concerning because it reduces the resilience of coral populations to withstand and recover from future bleaching events (Briggs et al. 2024).

Ultimately the results of this thesis can contribute to restoration initiatives, however, with the current ocean warming trend typical coral reefs may no longer function as before and small-scale restoration projects may not be enough to save coral reefs globally (Hoegh-Guldberg et al. 2023; Hughes et al. 2023; Lachs et al. 2023). Furthermore, although local management of water quality and fishing practices are required to conserve corals the major threat of global climate change needs to be managed (Richards 2018; Schoepf et al. 2019; Jung et al. 2021; Couce et al. 2023). To limit global-scale coral reef loss and by extension 25% of ocean

biodiversity and the livelihoods of more than half a billion people, climate change has to be limited with concerted effort towards reducing global greenhouse gas emissions (Hoegh-Guldberg et al. 2023).

6.3 Conclusion

Reducing global greenhouse gas emissions is the primary way to limit mass bleaching events. However, understanding the physiological mechanisms that drive coral resilience to thermal stress can contribute towards conservation measures that can aid in the protection of coral reefs. This thesis provides an in-depth account of the physiological mechanisms that drive coral resilience to thermal stress of marginal corals from extreme habitats. Overall, the long-term laboratory and field study found differential thermal resilience between habitat and species. Physiological plasticity and maintenance of tissue content were found to influence coral resilience to thermal stress. This thesis highlights the need to investigate extreme and marginal populations to attain insights into thermal resilience mechanisms and that these populations need to be added to conservation measures.

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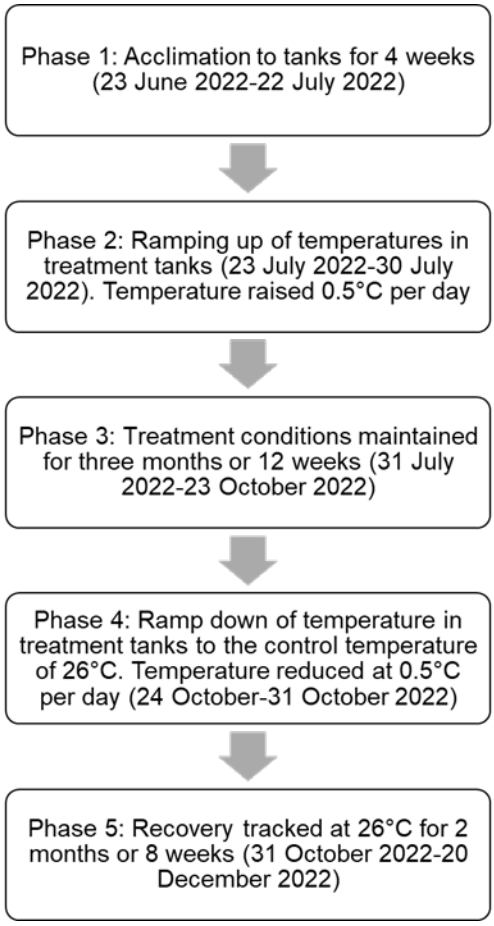
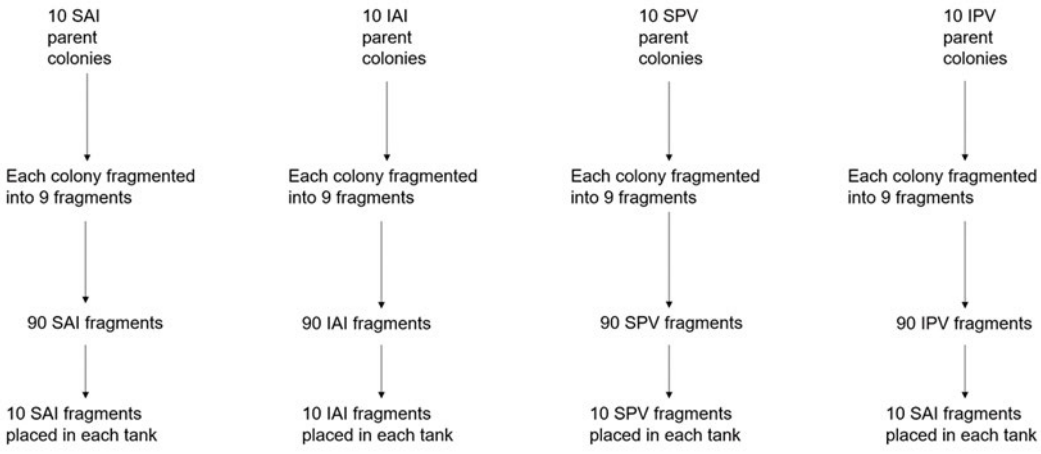
Appendices

a



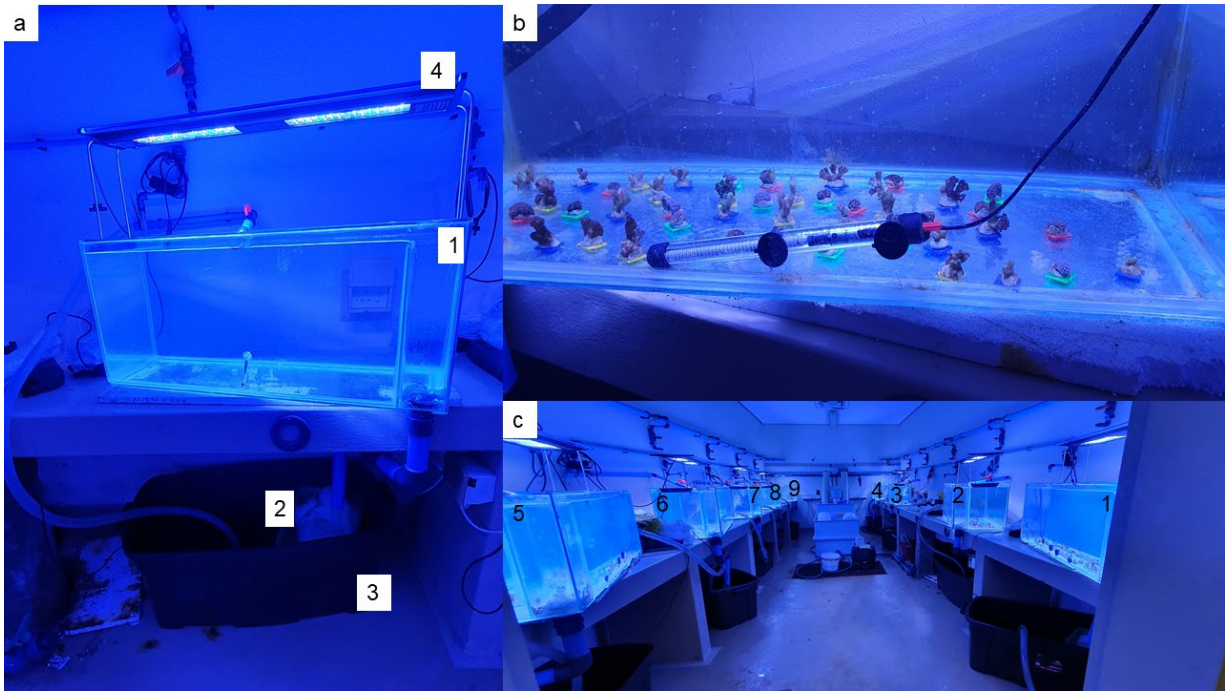
b



c

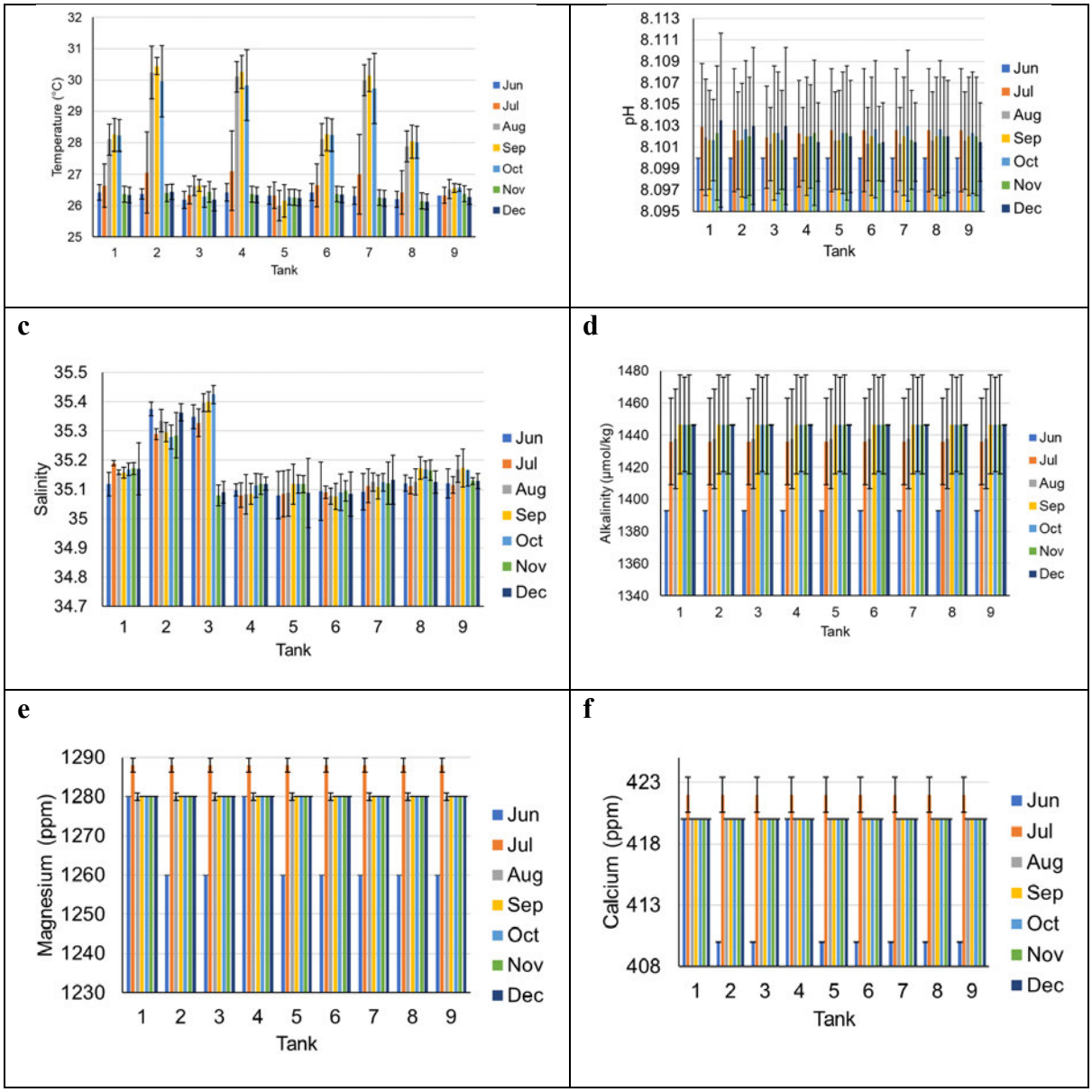
Variable	Sampling time points
coral score	31 Jul
	05 Aug
	12 Aug
	19 Aug
	25 Aug
	31 Aug
	09 Sep
	16 Sep
	23 Sep
	30 Sep
	07 Oct
	14 Oct
	23 Oct
	28 Oct
	04 Nov
	11 Nov
	18 nov
	27 Nov
	02 Dec
	09 Dec
	16 Dec
	20 Dec
respiration, photosynthetic, and growth rates	23july
	31 Jul
	31 Aug
	30 Sep
	23 Oct
	27 Nov
	20 Dec
sacrifice for tissue extractions	31 Jul
	23 Oct
	20 Dec

Appendix 1: **a** Phase flow diagram showing the timeline of the study and **b** Experimental tree diagram showing the number of fragments attained from each colony for both species for both habitats that were used in this study (SAI denotes subtidal *Anomastreae irregularis*, IAI denotes intertidal *Anomastreae irregularis*, SPV denotes subtidal *Pocillopora verrucoasa*, and IPV denotes intertidal *Pocillopora verruocsa*) **c** The sampling time points for each variable measured during the study



Appendix 2: **a1** One of nine 160 L main aquarium tank used to house the corals. Seawater from the main aquarium tank flowed through **a2** Mechanical filter which was a basket with cotton batting into **a3** 150 L sump that housed biological filter which was 2 Kg of lava rock and a recirculation pump (X5000 EnjoyRoyal, China (4000l/h)) equipped with a sponge filter that pumped the seawater back up to the main aquarium tank. Each aquarium tank was also equipped with **a4** One aquarium light (ZT6600II Zetlight, China) to provide illumination daily for 12 hours. **b** A picture of one aquarium tank with the glass submersible heater and the coral fragments, subtidal *Anomastraea irregularis* on the red tiles, intertidal *Anomastraea irregularis* on the green tiles, subtidal *Pocillopora verrucosa* on the yellow tiles and intertidal *Pocillopora verrucosa* on the blue tiles. **c** A picture of the air-conditioned marine science unit room with the 9 tanks running, the tanks numbers denoted **c1** The temperature treatment 1 (28°C) replicate 1, **c2** The temperature treatment 2 (30°C) replicate 1, **c3** The control (26°C) replicate 1, **c4** The temperature treatment 2 replicate 3, **c5** Control replicate 2, **c6** Temperature treatment 1 replicate 2, **c7** Temperature treatment 2 Replicate 2, **c8** Temperature treatment 1 replicate 3, and **c9** Control replicate 3.

a	**b**



Appendix 3: **a** The average ± 1 standard deviation temperature recorded by the ibutton in each tank every 30 minutes from the start until the end of the study. Tank 1 is the temperature treatment 1 replicate 1, tank 2 is the temperature treatment 2 replicate 1, tank 3 is the control replicate 1, tank 4 is the temperature treatment 2 replicate 3, tank 5 is control replicate 2, tank 6 is temperature treatment 1 replicate 2, tank 7 is temperature treatment 2 replicate 2, tank 8 is temperature treatment 1 replicate 3, and tank 9 is control replicate 3. **b** The average ± 1 standard deviation pH recorded in each tank daily from the start until the end of the study. **c** The average ± 1 standard deviation salinity recorded in each tank daily from the start until the end of the study. **d** The average ± 1 standard deviation alkalinity (dKh) recorded in each tank daily from the start until the end of the study. **e** The average ± 1 standard deviation magnesium (ppm)

recorded in each tank daily from the start until the end of the study. **f** The average ± 1 standard deviation calcium (ppm) recorded in each tank daily from the start until the end of the study.

Appendix 4: Bonferroni pairwise comparisons showing when the coral scores of the *Anomastraea irregularis* and *Pocillopora verrucosa* fragments significantly differed during the study

Bonferroni pairwise results table

Appendix 5: Bonferroni pairwise comparisons showing when the respiration, gross photosynthetic rates, and P:R ratios of the *Anomastraea irregularis* and *Pocillopora verrucosa* fragments significantly differed during the study

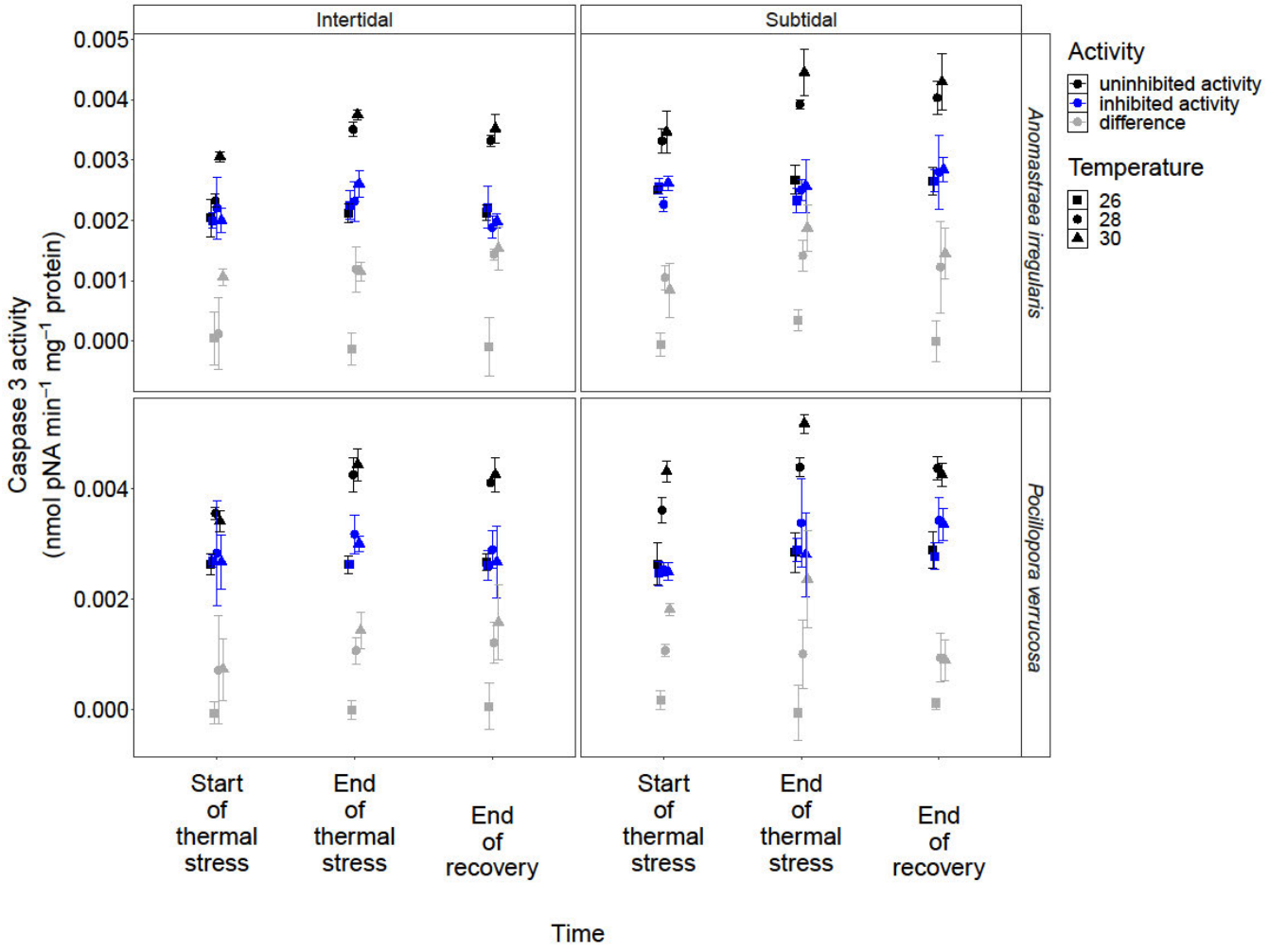
Bonferroni pairwise results tables

Appendix 6: Bonferroni pairwise comparisons showing when the growth rates of the *Anomastraea irregularis* and *Pocillopora verrucosa* fragments significantly differed during the study

Bonferroni pairwise results tables

Appendix 7: Bonferroni pairwise comparisons showing when the tissue content of the *Anomastraea irregularis* and *Pocillopora verrucosa* fragments significantly differed during the study

Bonferroni pairwise results tables



Appendix 8: The average ± 1 standard deviation non-inhibited, inhibited, and difference between the inhibited and non-inhibited caspase 3 activity for both species from both habitats at different points during the experiment

Appendix 9: Bonferroni pairwise comparisons showing when the protein concentration of the *Anomastraea irregularis* and *Pocillopora verrucosa* fragments significantly differed during the study

Bonferroni pairwise results table

Appendix 10: Bonferroni pairwise comparisons showing when the superoxide dismutase activity of the *Anomastraea irregularis* and *Pocillopora verrucosa* fragments significantly differed during the study

Bonferroni pairwise results table

Appendix 11: Bonferroni pairwise comparisons showing when catalase activity of the *Anomastraea irregularis* and *Pocillopora verrucosa* fragments significantly differed during the study

Bonferroni pairwise results tables

Appendix 12: Bonferroni pairwise comparisons showing when the glutathione peroxidase activity of the *Anomastraea irregularis* and *Pocillopora verrucosa* fragments significantly differed during the study

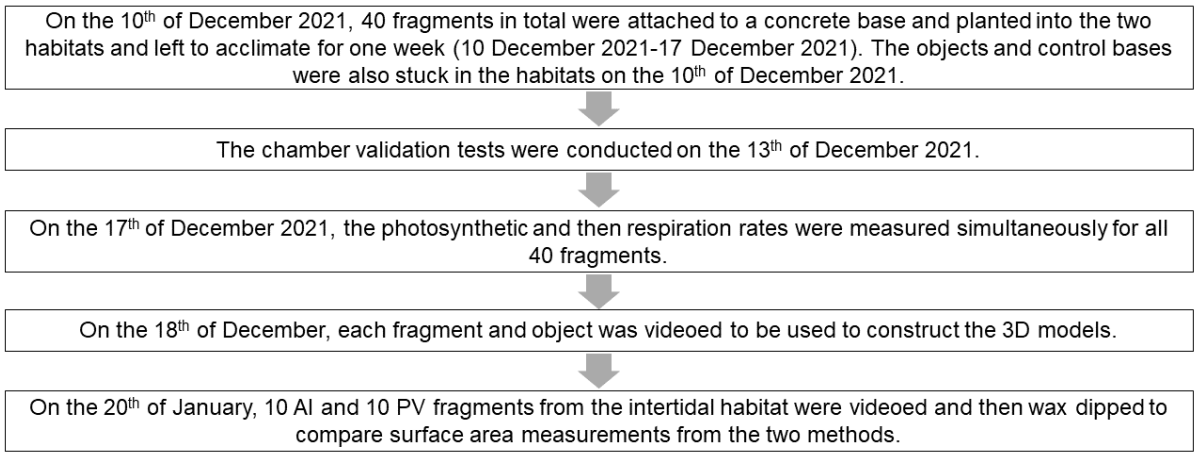
Bonferroni pairwise results table

Appendix 13: Bonferroni pairwise comparisons showing when the glutathione S-transferase activity of the *Anomastraea irregularis* and *Pocillopora verrucosa* fragments significantly differed during the study

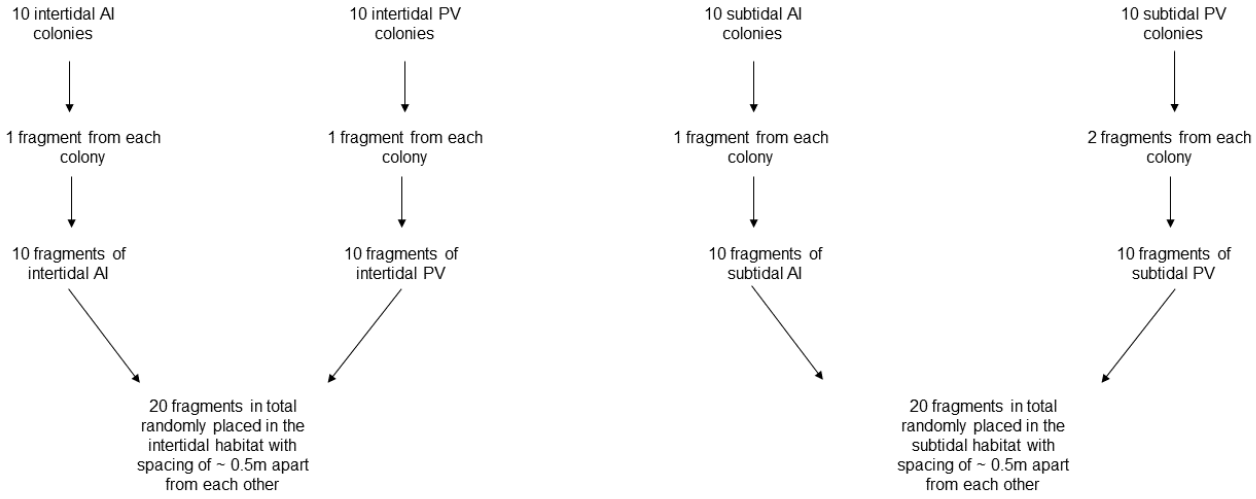
Bonferroni pairwise results table

Appendix 14: Bonferroni pairwise comparisons showing when the caspase 3 activity of the *Anomastraea irregularis* and *Pocillopora verrucosa* fragments significantly differed during the study

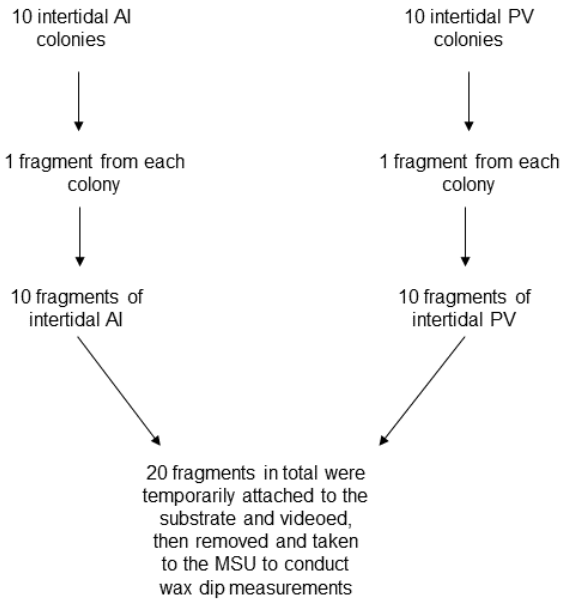
Bonferroni pairwise results table



Appendix 15: Flow diagram showing when sampling was conducted



Appendix 16: Experimental tree diagram showing the number of fragments attained from each colony for both species for both habitats that were used for the metabolic rates and surface area measurements

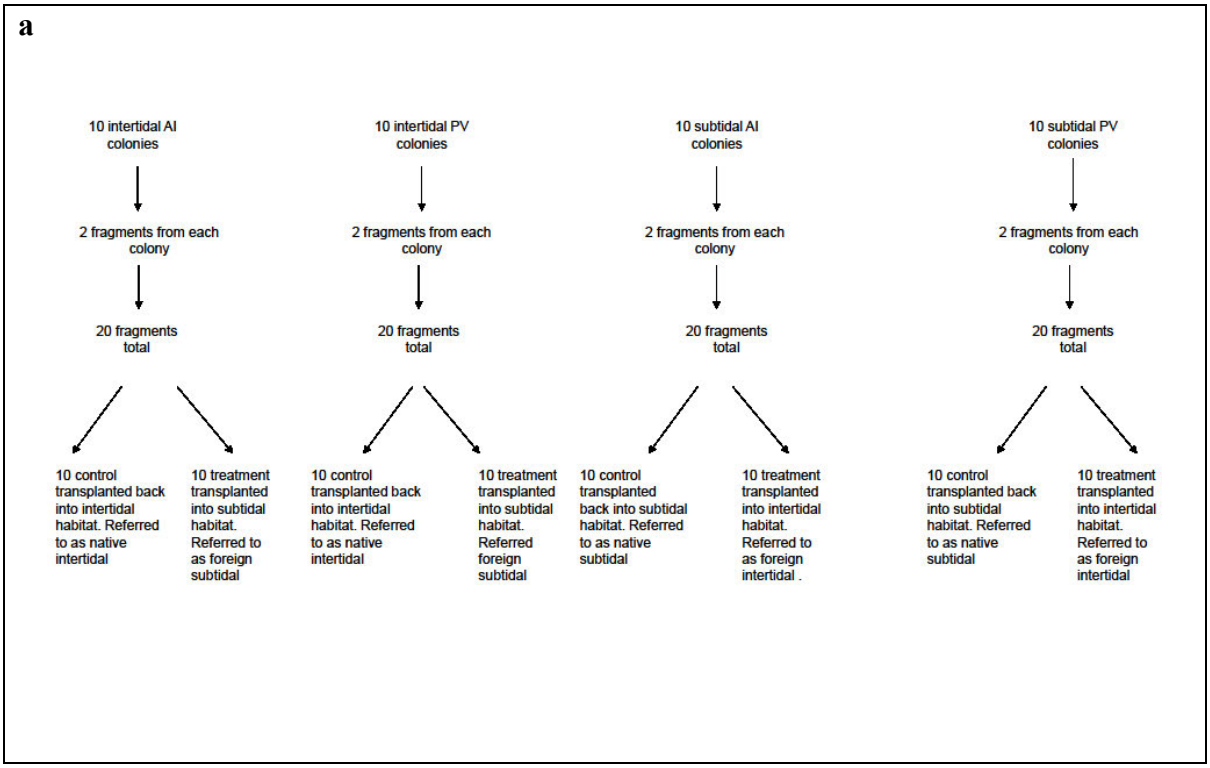


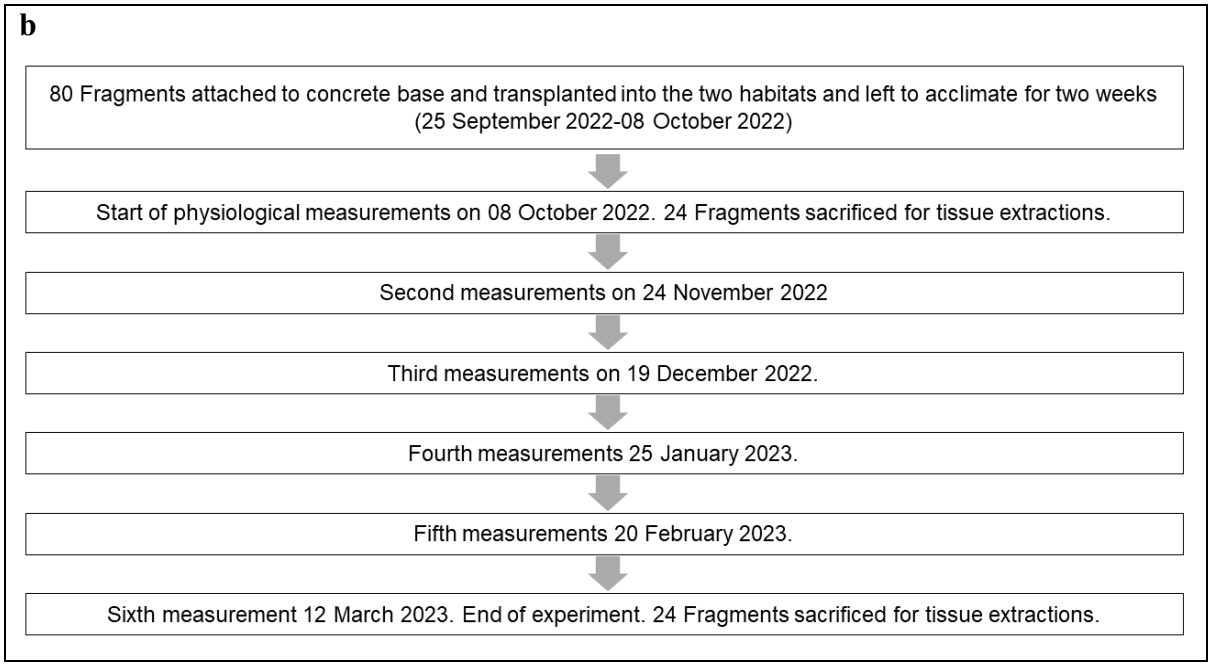
Appendix 17: Experimental tree diagram showing the number of fragments attained from each colony for both species from the intertidal habitat that were used to attain surface area measurements to compare the 3D photogrammetry method to the wax dip method

Appendix 18: Results of the Bonferroni pairwise comparisons for the respiration rate, photosynthetic rate, and surface area of the coral fragments. AI denotes *Anomastraea irregularis*, PV denotes *Pocillopora verrucosa*, and * indicates significant differences.

Variable	(A) species x habitat	(B) species x habitat	Mean Difference (A-B)	Standard Error	df	p
respiration rate	AI x intertidal	AI x subtidal	-0.0009	0.00032	1	0.022*
		PV x intertidal	0.0091	0.00032	1	<0.0005*
		PV x subtidal	0.0089	0.00032	1	<0.0005*
	AI x subtidal	PV x intertidal	0.01	0.00032	1	<0.0005*
		PV x subtidal	0.0098	0.00032	1	<0.0005*
	PV x intertidal	PV x subtidal	-0.0002	0.00032	1	0.998
photosynthetic rate	AI x intertidal	AI x subtidal	0.0013	0.00092	1	0.946
		PV x intertidal	-0.004	0.00092	1	<0.0005*
		PV x subtidal	-0.0026	0.00092	1	0.034*
	AI x subtidal	PV x intertidal	-0.0053	0.00092	1	<0.0005*
		PV x subtidal	-0.0039	0.00092	1	<0.0005*
	PV x intertidal	PV x subtidal	0.0014	0.00092	1	0.737
surface area	AI x intertidal	AI x subtidal	0.741	0.60029	1	0.998
		PV x intertidal	-25.566	0.60029	1	<0.0005*
		PV x subtidal	-25.775	0.60029	1	<0.0005*
	AI x subtidal	PV x intertidal	-26.307	0.60029	1	<0.0005*
		PV x subtidal	-26.516	0.60029	1	<0.0005*
	PV x intertidal	PV x subtidal	-0.209	0.60029	1	0.998

a





Appendix 19: **a** Experimental tree diagram for the experiment AI denotes *Anomastraea irregularis* and PV denotes *Pocillopora verrucosa* total n = 80 (10 colonies x 2 replicate fragments x 2 species x 2 habitats), **b** Phase flow diagram showing when sampling was conducted during the study

Appendix 20: Overview of the percentage survival of transplanted coral fragments in the intertidal and subtidal habitats for each species (II= native intertidal, SS= native subtidal, IS=intertidal fragments transplanted into the subtidal habitat, SI= subtidal fragments transplanted into the intertidal habitat)

		Origin	Starting number of fragments	Number of fragments Sacrificed at start	remaining Number of fragments at start	Number of dead fragments after 2 months	Remaining number of fragments after two months	Number of fragments dead after 3 months	Remaining number of fragments after 3 months	Number of fragments missing/lost	Final number of fragments remaining	Taking sacrifice and lost frags into considerati on	%survival at the end
Species	Pocillopora verrucosa	II	10	3	7	0	7	0	7	1	6	6	100
		SI	10	3	7	1	6	1	5	0	5	7	71
		SS	10	3	7	0	7	0	7	1	6	6	100
		IS	10	3	7	0	7	0	7	1	6	6	100
	Anomastraea irregularis	II	10	3	7	0	7	0	7	2	5	5	100
		SI	10	3	7	0	7	1	6	0	6	7	86
		SS	10	3	7	0	7	0	7	1	6	6	100
		IS	10	3	7	0	7	0	7	0	7	7	100

Appendix 21: Bonferroni pairwise comparisons showing when the coral scores of the coral fragments significantly differed during the study

Bonferroni pairwise results table

Appendix 22: Bonferroni pairwise comparisons for respiration rates, gross photosynthetic rates, and P:R ratios of the *Anomastraea irregularis* and *Pocillopora verrucosa* fragments between Origin and Habitat

Bonferroni pairwise results table

Appendix 23: Bonferroni pairwise comparisons showing when the growth rates of the *Anomastraea irregularis* and *Pocillopora verrucosa* fragments significantly differed during the study and between Origin and Habitat

Bonferroni pairwise results table

Appendix 24: Bonferroni pairwise comparisons showing when the Symbiodiniaceae cell density in the coral fragments significantly differed during the study and between Origin and Habitat

Bonferroni pairwise results table

Appendix 25: Bonferroni pairwise comparisons showing when the chlorophyll-a concentration in the coral fragments significantly differed during the study and between Origin and Habitat

Bonferroni pairwise results table

Appendix 26: Bonferroni pairwise comparisons showing when the chlorophyll-a per Symbiodiniaceae cell in the coral fragments significantly differed during the study and between Origin and Habitat

Bonferroni pairwise results table

Appendix 27: Bonferroni pairwise comparisons showing when the lipid content in the coral fragments significantly differed during the study and between Origin and Habitat

Bonferroni pairwise results table